EXTERNAL SCIENTIFIC REPORT

Survey on sterigmatocystin in food[1]

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ABSTRACT

A total of 1 259 samples of cereal grains, cereal products, beer and nuts were analysed for the presence of the mycotoxin sterigmatocystin. Samples were mainly collected at processing plants, storage facilities, wholesale and retail between August 2013 and November 2014, in nine European countries (mostly Greece, Italy, the Netherlands and the United Kingdom (UK), additionally in Cyprus, Germany, Latvia, Lithuania, and Poland). The products originated from 27 European countries and 18 other countries (mostly rice and nuts). The samples comprised cereal grains (221 wheat, 35 rye, 33 maize, 59 barley, 51 oats, 2 spelt, 117 rice), grain milling products (125), pasta (115), bread/rolls (143), breakfast cereals/muesli (97), fine bakery ware (90), cereal-based infant food (54), beer (53), peanuts (28) and hazelnuts (36). All samples were analysed by methods based on liquid chromatography with tandem mass spectrometry (LC-MS/MS). The limit of quantification, defined as the lowest validated level, was 0.5 µg/kg and the limit of detection (LOD) was in the range 0.05–0.15 µg/kg (0.005–0.01 µg/kg for beer). Overall, sterigmatocystin was identified in 10 % of the samples. More than 50 % of the contaminated samples contained levels between LOD and 0.5 µg/kg. In the other cases levels were all in the range 0.5–6 µg/kg with one exception (33 µg/kg in oats). Rice and oats were identified as the cereals most prone to sterigmatocystin contamination (virtually all unprocessed rice samples (all from Europe), 21 % of the processed rice samples, 22 % of the oats grains). In cereal products, levels were typically lower than in grains. The highest incidence was in breakfast cereals/muesli (19 %) while for the other cereal products the detection rate was 5–7 %. In the contaminated cereal products, rice and oats were often present as ingredients. For beer and nuts, none of the samples were found to be contaminated with sterigmatocystin.

KEY WORDS

sterigmatocystin, survey, occurrence, food, cereals, beer, nuts

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SUMMARY

Sterigmatocystin is a mycotoxin structurally related to aflatoxin B_1 that can occur in grains and grain-based products as well as certain other food products due to fungal infestation especially at the post-harvest stage. Due to lack of suitable data on the occurrence of sterigmatocystin, the EFSA Panel on Contaminants in the Food Chain (CONTAM Panel) could not characterise its risk for human health. A major problem was the sensitivity of the methods applied. In March 2013 a call for proposal was published to generate such data.

This report describes the outcome of project CP/EFSA/CONTAM/2013/02 'Survey on sterigmatocystin in food' carried out in accordance with Article 36 of Regulation (EC) No 178/2002, which was designed to obtain representative data on the occurrence of sterigmatocystin in Europe, using validated state-of-the-art analytical methods.

Analytical methods based on liquid-chromatography with tandem mass spectrometry (LC-MS/MS) were used for the determination of sterigmatocystin in cereal grain, cereal products (including beer), and nuts, by four laboratories. The methods were validated down to 0.5 µg/kg in each of the matrices and met the requirements as laid down in Commission Regulation (EC) No 401/2006. Limits of detection in the range of 0.05–0.15 µg/kg for grain, cereal products and nuts, and 0.005–0.01 µg/kg for beer were achieved. The comparability of the results generated by the four laboratories was demonstrated. Method performance was adequate throughout the analysis of the survey samples.

A total of 1 259 samples of cereal grains, cereal products, and nuts, collected between August 2013 and November 2014 in nine European countries and originating from 45 countries, were analysed for the presence of sterigmatocystin.

Sterigmatocystin was detected in 10 % of all samples. In more than 50 % of these samples the level was below 0.5 µg/kg. Sterigmatocystin exceeded 1.5 µg/kg in 1.4 % of all food samples analysed in this survey.

In cereal grains sterigmatocystin was detected in 2–6 % of the wheat, rye, maize, and barley samples, mostly at levels below 1.5 µg/kg. A higher incidence and higher levels of contamination (14 samples 1.5–6 µg/kg, one 33 µg/kg) were observed for rice (virtually all unprocessed rice from EU, 21 % of the processed rice) and oats (22 %).

In grain milling products and pasta, sterigmatocystin was detected in 5 % of the samples, mostly below 0.5 µg/kg. In bread, fine bakery ware, and cereal-based infant food, sterigmatocystin was found in 7 % of the samples, and in breakfast cereals more frequently (19 %). From the samples belonging to these four types of products that are consumed as such, 11 samples (2.9 %) were contaminated at 0.5–1.5 µg/kg and 3 (0.8 %) at 1.5–5 µg/kg.

Sterigmatocystin was not detected in any of the peanut, hazelnut and beer samples analysed in this survey.

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BACKGROUND AS PROVIDED BY EFSA

EFSA's Panel on contaminants in the food chain (CONTAM Panel) assessed the risk for public and animal health related to the presence of sterigmatocystin in food and feed (EFSA CONTAM Panel, 2013).

Sterigmatocystin is a mycotoxin which is structurally related to aflatoxin B_1. Occurrence of sterigmatocystin has been reported in grains and grain-based products, green coffee beans, beer, spices, nuts, cheese and feed. The occurrence data available in literature were mostly obtained with methods that are not state of the art and show a high percentage of left-censored data. However, recently a sensitive liquid chromatography tandem mass spectrometry (LC-MS/MS) method has become available for the analysis of sterigmatocystin in grains, grain-based products and cheese.

Sterigmatocystin is a genotoxic carcinogen and therefore risk characterisation should be based on the margin of exposure (MOE) approach. However, the absence of representative exposure data for the European population precludes the application of the MOE approach.

Therefore, the present call for proposals aims at the preparation of a study on the occurrence of sterigmatocystin in food with special focus on grains and grain based products for human consumption from different geographic regions in Europe, to possibly serve as supporting information to the CONTAM Panel for future exposure assessment for sterigmatocystin.

TERMS OF REFERENCE AS PROVIDED BY EFSA

This call for proposals aims at obtaining representative occurrence data of sterigmatocystin in food samples with special focus on grains and grain-based products for human consumption from different geographic regions in Europe by using a state of the art method such as LC-MS/MS. The beneficiary shall perform the following tasks, in order to achieve the objectives:

1. To elaborate a protocol for collecting the samples that is in accordance with the Commission Regulation (EC) No 401/2006[2] and that shall take into account the following requirements;

    a. the samples shall be taken from at least three different European countries (preferably not from neighbouring countries);

    b. the following grains for human consumption and food products shall be analysed:

        i. at least 400 samples of grains including wheat, barley, rye, oats and rice;

        ii. at least 500 samples of grain-based products for human consumption including flour, bread and rolls, pasta, cereal flakes and muesli;

        iii. the inclusion of at least 50 samples of nuts for human consumption is considered as an asset;

        iv. the inclusion of at least 50 samples of beer is considered as an asset.

2. To collect the samples;

[2] Commission Regulation (EC) No 401/2006 of 23 February 2006 laying down methods of sampling and analysis for the official control of the levels of mycotoxins in foodstuffs. Official Journal of the European Union. 9.3.2006, L70, 12–34.

3. To analyse the collected samples using a state of the art method such as LC-MS/MS that complies with the requirements of the Commission Regulation (EC) No 401/2006 and more precisely with the performance criteria for aflatoxin B_1 and that has a sensitivity comparable to methods that have recently been published in the literature;

4. To prepare a Final External Scientific Report and a database providing the results of the analyses performed for food samples intended for human consumption. The database as well as the interim and Final External Scientific Reports will be prepared in line with the time schedule reported in the call for proposals.

The Final as well as the Interim Scientific reports shall be written in English and follow the template structure provided by EFSA and EFSA citation standards. The External Scientific Report shall contain the following information: the justification of the choice and the description of the analytical method applied; the validation results of the method for all analysed matrices (similar matrices can be combined for the validation however reasoning shall be provided); the description of the sampling procedure applied; the results of the individual samples; common statistical descriptors (e.g. mean, median, standard deviation) of the concentrations; a critical evaluation of the reliability of the submitted data and the related uncertainties, e.g. in the analytical methods, sampling, etc.

The database shall be written in English and shall follow the EFSA Guidance on standard sample description and should be submitted via the Evidence management Unit's (DATA) call for continuous collection of chemical contaminants occurrence data in food and feed. It shall contain the following information: the concentrations of sterigmatocystin in the analysed samples, associated information describing the sample and the other sample description details specified in the most recent EFSA Guidance on standard sample description.

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- Food and Environment Research Agency (Fera), York, UK
- Benaki Phytopathological Institute (BPI), Kifissia, Greece
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INTRODUCTION AND OBJECTIVES

Sterigmatocystin is a mycotoxin that is produced by more than 50 fungal species, including *Aspergillus flavus*, *A. parasiticus*, *A. nidulans, and A. versicolor*. The latter is the most common source. Sterigmatocystin shares its biosynthetic pathway with aflatoxins (EFSA CONTAM Panel, 2013). *A. nidulans* and *A. versicolor* seem unable to metabolise sterigmatocystin into O-methylsterigmatocystin, the direct precursor of aflatoxin B_1 and G_1. As a consequence, food commodities infested by these fungi can contain high amounts of sterigmatocystin, whereas infestation by *A. flavus* and *A. parasiticus* results in low amounts of sterigmatocystin because most is converted into aflatoxins (Sweeney and Dobson, 1999; Yu et al., 2004; Rank et al., 2011).

Sterigmatocystin can occur in grains and grain-based products due to fungal infestation especially at the post-harvest stage. Furthermore, sterigmatocystin has been reported to occur in green coffee beans, spices, nuts and beer, and on the surface of cheese during ripening and storage (Battilani et al., 2009; Veršilovskis and De Saeger, 2010). Milling, baking, roasting, and other food processing steps tend to decrease the sterigmatocystin concentration (Takahashi et al., 1984; Bokhari and Aly, 2009; Veršilovskis and Bartkevics, 2012).

The number of surveys on occurrence of sterigmatocystin in food performed so far have been rather limited and were partly carried out in the 1970s-1980s with less sophisticated techniques and relatively high limits of detection (LODs), often in the range 20–50 µg/kg. Since the late 1990s more advanced techniques (LC-MS) have been employed with lower LODs. Although in some studies using LC-MS methods no sterigmatocystin was detected (e.g. UK, Scudamore et al., 1996; FSA 2001), Veršilovskis et al. (2008a) reported the occurrence in 55 out of 215 samples from Latvia, with 24 samples in the range 25–83 µg/kg. More recently, sterigmatocystin has been included in LC-MS/MS-based multi-mycotoxin methods and measured concurrently with the determination of other mycotoxins. This has resulted in findings in indoor (non-food) matrices (Vishwanath et al., 2009), mouldy food (private household conditions) (Sulyok et al., 2010), cereal grains (Uhlig et al., 2013), nuts (Varga et al., 2013) and spices (Yogendrarajah et al., 2014). Although the benefits of LC-MS/MS are fully recognised, the technique is still in the process of being adopted and implemented in monitoring and control laboratories. Consequently, a call for data on sterigmatocystin launched by EFSA in 2010 resulted in only a limited number of results for food samples (247) which were mostly left censored (< LOD). Due to the lack of data EFSA could not characterise the risk of sterigmatocystin for human health (EFSA CONTAM Panel, 2013).

The main objective of this study is to provide representative data on the occurrence of sterigmatocystin in both raw agricultural commodities and in processed food products within the Member States of the European Union, which can be used as supporting information to the EFSA Panel on Contaminants in the Food Chain (CONTAM Panel) for exposure assessment for this mycotoxin. This is done by sampling food commodities and products, with emphasis on grains and cereal-based products for human consumption, from different geographic regions in Europe. The samples are analysed using state-of-the-art validated LC-MS/MS methodology enabling quantitative determination at ≤ 0.5 µg/kg. This is more than one magnitude lower than in the majority of the currently existing surveys and also below the recommended limit of quantification (LOQ) of 1.5 µg/kg (EFSA CONTAM Panel, 2013).

MATERIALS AND METHODS

1. Sampling plan

The aim of this first extensive European survey on sterigmatocystin in food was to gain insight in the occurrence of sterigmatocystin in cereals and cereal-based products as consumed in the EU. The products and their numbers to be included in the survey as specified in the EFSA call were: at least 400 samples of grains including wheat, barley, rye, oats and rice, and at least 500 samples of grain-based products for human consumption including flour, bread and rolls, pasta, cereal flakes and muesli. Furthermore, additional samples of beer (50) and nuts (50) were desirable. In the products to be sampled, two categories were distinguished: cereal grains (the raw agricultural commodities), and processed cereal products.

Within the scope specified by EFSA, the starting point with respect to the sampling strategy was objective sampling. Therefore the initial sampling plan was largely consumption oriented. For cereal grains within the EU, wheat is by far the most important with respect to intake (70 %), followed by rye, maize, rice, barley, and oats (in decreasing order from 8 % to 1.5 %) (Herrmann et al., 2011). For this reason, the emphasis in sampling of cereal grains was on wheat. For the five minor grains the number of samples in the sampling plan was set at a fixed minimum in order to avoid that results for e.g. oats would be based on a very limited number of samples only. Another reason to do so was because the relative importance of the minor grains may vary per country or consumer group (e.g. for coeliac patients where maize and rice would be more relevant). The anticipated sample numbers per type of cereal are given in Table 1.

The processed cereal products were divided into two subgroups: food ingredients that are typically further processed by e.g. baking or cooking, and products that are consumed as such without further processing. The choice of product categories and numbers within each category was based on intake estimations derived from food consumption surveys (Van Rossum et al., 2011; EFSA, 2014) which were discussed with EFSA and fine-tuned at the start of the project. For nuts, given the limited number of samples (50), a higher number of samples for only two commodities was preferred over low sample numbers for more commodities. Besides peanuts, by far the most consumed nut, hazelnuts were selected as second nut (highest consumption amongst tree nuts). The resulting anticipated numbers per category of processed cereal products and nuts are included in Table 1.

For the different type of products, a target of approximately 5 % of organic produce was aimed for during sample selection and sampling.

In order to be representative for the situation in the European Union with respect to production, climate, storage practices, dietary habits, and to cover domestic as well as imported cereals and cereal products, the sampling plan involved sampling in different countries in different regions within the European Union, i.e. the United Kingdom, the Netherlands (including sampling events in Germany), Italy, Greece and Cyprus. During the kick-off meeting of the project it was concluded that it was desirable to include samples from Eastern Europe and this was therefore foreseen in the finally adopted sampling plan. With this, since part of the products sampled will originate from other countries than the country of sampling, the overall coverage was considered sufficiently representative for the purpose of this survey.

Table 1: Summary of sampling plan

Product	Number of samples
Cereal grains	**400**
Wheat	200 (160 soft and 40 hard/durum)
Rye	40
Maize	40
Rice	40
Barley	40
Oats	40
Cereal products	**500**
Products consumed with further processing	
Grain milling products	60
Rice	40
Pasta	100
Products consumed without further processing	
Bread and rolls	150
Breakfast cereals (incl. muesli)	50
Fine bakery ware	60
Cereal-based infant food	40
Beer	**50**
Nuts	**50**
Peanuts	25
Hazelnuts	25

2. Sampling

Samples were taken at various points in the food chain including primary production, storage, import activities, border inspection activities, processing plants (mills), wholesale, and retail sale. Samples were taken by official governmental inspection bodies (UK: Food Standards Agency; the Netherlands: Food and Consumer Product Safety Authority (NVWA); Greece: Regional Center of Plant Protection and Quality Control of Piraeus, Regional Center of Plant Protection and Quality Control of Thessaloniki, Directorate of Rural Economy & Veterinary/Department of Quality & Phytosanitary Control/Metropolitan Unity of Thessaloniki/Region of Central Macedonia) and by third parties (Food Allergens Laboratory, Greece/Cyprus), cereal trade bodies and food industry collaborators. At the retail level, products were also sampled by staff of the analytical laboratories themselves. Information on requirements for official sampling as well as samples sheets to obtain detailed sample information was provided to third parties (for example see Appendix 1). In the majority of the cases, sampling was done according to the guidelines for the official control of foodstuffs as described in Commission Regulation (EC) No 401/2006. Bulk products were sampled using the appropriate sampling probes or suction devices that are also used for aflatoxin monitoring and control. In case of streaming lots, incremental samples at several time intervals were collected. For packed products, depending on the size of one unit, sampling probes were used or the appropriate number of units were sampled and combined to achieve the required amount of aggregate sample. Depending on the product and the size of the lot sampled, the amount of aggregate sample varied from 1 to 20 kg.

Upon receipt in the analytical laboratory, samples were given a unique code. If not immediately homogenised, samples were stored under dry conditions at controlled temperature. Raw cereals and dry products (pasta, flour) at approximately 20 °C, high moisture products (bread, rolls) at 4 °C. The

time between storage and homogenisation was not more than two days. Packed products were stored at ambient conditions unless the label specified otherwise and processed before the 'best before' date.

3. Analytical method

Sample analysis of cereals and cereal products was performed by four laboratories: RIKILT – Wageningen UR (the Netherlands), Università Cattolica del Sacro Cuore (UCSC, Italy), Food and Environment Research Agency (Fera, UK), and Benaki Phytopathological Institute (BPI, Greece). Given the limited number of samples for beer and nuts, these analyses were performed by one laboratory (UCSC and BPI, respectively). All laboratories had experience with LC-MS/MS-based methods for the determination of mycotoxins, including sterigmatocystin. For cereals, cereal products and nuts, an extraction based on acetonitrile/water was chosen from the different suitable options (Sulyok et al., 2007; Mol et al., 2008; Spanjer et al., 2008; Veršilovskis et al., 2010; Lacina et al., 2012) because it is a very commonly used extraction solvent for sterigmatocystin as well as many other mycotoxins. Although no reference material with naturally contaminated sterigmatocystin is yet available, and, therefore, the extraction yield of sterigmatocystin cannot be fully assessed, the suitability of this extraction solvent has been demonstrated for the structurally related aflatoxins in reference materials. LC-MS/MS was the preferred methodology for sample analysis because of the high sensitivity and selectivity. A potential complication with the use of LC-MS/MS is the fact that matrix components co-eluting with sterigmatocystin may affect the ionisation efficiency. This may lead to ion suppression (or enhancement) of the response obtained for sterigmatocystin in samples compared to solvent standards and consequently may affect the quantification. This type of matrix effects can be compensated most effectively by the use of isotopically labelled sterigmatocystin as internal standard. A suitable label was commercially available and by normalising the response of sterigmatocystin for its labelled internal standard it is possible to use solvent standards for calibration for the different products analysed.

For the purpose of this survey, the existing (multi-mycotoxin) methods were adapted for optimal performance for sterigmatocystin, analysis time, and/or to achieve the low limits of identification and quantification required for this survey (≤ 1.5 µg/kg (EFSA CONTAM Panel, 2013)). Besides the use of acetonitrile/water for extraction, the use of LC-MS/MS for analysis of the extracts, and the use of isotopically labelled sterigmatocystin for quantification, the methods employed in the four laboratories were not standardized in further detail. The laboratories were allowed to use the method considered optimum for their situation and LC-MS/MS instrumentation, as long as the performance requirements as laid down for aflatoxin B_1 in Commission Regulation (EC) No 401/2006 were met.

3.1. Chemicals and reagents

RIKILT: Chemicals and solvents used for sample preparation were 'pro-analysis' quality or better. Methanol, acetonitrile and LC–MS grade water were purchased from Biosolve (Valkenswaard, the Netherlands). Formic acid and ammonium formate were from Sigma-Aldrich (Zwijndrecht, the Netherlands). The analytical reference standard of sterigmatocystin was purchased as stock solution (50 mg/L in acetonitrile) from Biopure (Tulln, Austria). The internal standard U-[$^{13}C_{18}$]-sterigmatocystin (96.4 % ^{13}C) was also purchased as solution (26 µg/mL) (Biopure).

UCSC: Chemicals and solvents used for the extraction and clean-up were ACS grade or equivalent (Carlo Erba, Milan, Italy). Water used was deionised and purified through a Milli-Q treatment system (Millipore, London, UK). HPLC-MS grade acetonitrile, methanol, water and formic acid were purchased from Merck (Darmstadt, Germany). The composition of the phosphate buffer (PBS) was as follows: NaCl 8 g/L, KCl 0.2 g/L, Na_2HPO_4 1.15 g/L, KH_2PO_4 0.2 g/L; pH 7.4. The sterigmatocystin analytical reference standard was obtained from Sigma-Aldrich (St. Louis, MO, USA; purity 98.5 %);

The internal standard U-[$^{13}C_{18}$]-sterigmatocystin (96.4 % ^{13}C) standard solution (1.2 mL, 25.7 µg/mL, uncertainty 1.02 µg/mL) was purchased from Biopure (Tulln, Austria).

Fera: For extraction, acetonitrile (HPLC grade) (Sigma-Aldrich, Gillingham, UK) and water (18.2 MΩ/cm Purelab Ultra laboratory purification system) (Elga, Marlow, UK) were used. Methanol, acetonitrile, ammonium formate, formic acid, 99 % (UPLC/MS grade) (Biosolve, Dieuze, France via Greyhound, Birkenhead, UK) were used for eluent preparation for LC-MS/MS analysis. The analytical reference standard of sterigmatocystin was purchased as solid (dry film, Sigma Chemicals, ≥98 % purity). The internal standard U-[$^{13}C_{18}$]-Sterigmatocystin in acetonitrile was purchased from Romerlabs, but was sourced from Biopure (Tulln, Austria).

BPI: Stock solutions of sterigmatocystin (50.2 µg/mL) in acetonitrile and U-[$^{13}C_{18}$]-Sterigmatocystin internal standard (25.7µg/mL) were purchased from Romer Labs. The solutions were stored at -20 °C. Acetonitrile (Panreac Química S.L.U.), methanol and water (VWR International, LLC) were HPLC grade.

3.2. Homogenisation

For homogenisation of the aggregate sample (1–20 kg) either dry milling or the slurry method (Spanjer et al., 2006) was used as described below.

RIKILT: aggregate samples were homogenised by the laboratory of NVWA, located in the same building as the RIKILT laboratory. For 1–1.5 kg amounts of dry samples, flour was manually mixed and not further processed. Pasta was crushed and then milled (1 mm) using a centrifugal mill (Retsch ZM1000 or equivalent). Muesli and nut samples were homogenised in a kitchen mixer. Grains were first milled to 2 mm, then a portion (ca. 100 g) was further milled (1 mm) in a centrifugal mill. For samples > 1.5 kg in general, and for bread and fine bakery ware, the entire sample was transferred into a vessel and chlorine-free water was added in a ratio ranging from 1+1.5 to 1+3, depending on the product. Slurries were then prepared using a High Shearing mixer (Silverson EX) or Ultra Turrax, (model T 50, Janke & Kunkel). The homogenisation time depended on the size of the sample and the power of the mixing device. Sub portions of the homogenates were stored at < -18 °C when not immediately extracted.

UCSC: dry samples were milled (1 mm) using a centrifugal mill (Retsch); bread and rolls were dried at 65 °C overnight before milling. Muesli was homogenised in a kitchen mixer.

Fera: dry samples were homogenised by milling (0.5 or 1 mm) using a centrifugal mill (Retsch). For certain products (e.g. fruit-containing breakfast cereals) solid carbon dioxide was added during the milling process. In the case of some retail products, e.g. breakfast cereals such as those containing pieces of fruit and chocolate, some breads, fine bakery goods and ready to use noodles, a wet slurry preparation method as described by RIKILT (above) was used. Slurry choice and water ratio was dependent on the sample type and was carried out to ensure the final sample was adequately ground and homogenised. In all cases the full sample provided to the laboratory (range from 1 to 10 kg) was prepared. Sub portions of homogenates were stored at < -18 °C.

BPI: For 1 kg sample amounts, homogenization was performed in a food processor (Thermomix TM31, Vorwerk Group). For larger aggregate samples, the homogenization was performed in a dispersing homogenizer (Ultra Turrax, model T 65). In both cases, the entire sample was transferred into the mixing bowl and an appropriate amount of chlorine-free water was added in a ratio ranging

from 1+1.5 to 1+3, depending on the product. The homogenisation time depended on the size and nature of the sample. Homogenates were stored at < -18 °C when not immediately extracted.

3.3. Extraction and clean-up

RIKILT: for dry homogenates, 5 g was extracted with 20 mL of acetonitrile/water (80/20 v/v) by head-over-head shaking for 2 hours. In case of slurries, depending on the slurry composition, 7.5–10 g was extracted with 17.5–20 mL of acetonitrile by head-over-head shaking for 2 hours. After centrifugation, 500–650 µL of the clear supernatant was transferred into a mini-uniprep PTFE filter vial (0.45 µm) (Whatman, Buckinghamshire, UK), 50 µL of isotopically labelled sterigmatocystin in acetonitrile was added, and the volume was made up to 1.0 mL with water. After vortex mixing, the extracts were filtered in-vial by press-through. The amount of matrix equivalent in the final extract was 0.0625 g sample/mL, i.e. sample preparation involved a dilution of the sample by a factor of 16. The concentration of internal standard added to the extract corresponded to 1.5 µg/kg on sample basis.

UCSC: sterigmatocystin was extracted from 25 g of milled sample using 100 mL acetonitrile-water 80+20 v/v for 60 minutes. After filtration through a folded filter paper, 5 mL of filtrate was diluted with 20 mL of phosphate buffer (PBS, pH 7.4) and cleaned using a solid phase extraction (SPE) column (OASIS HLB, 500 mg; Waters). After washing of the column with subsequently 2 mL of water and 2 mL of water-methanol 75+25 v/v, sterigmatocystin was eluted in a graduated glass vial with 6 mL acetonitrile. The extract was concentrated under a gentle flow of nitrogen and brought to 2 mL with acetonitrile-water 40+60 v/v. The amount of matrix equivalent in the extract obtained this way was 0.625 g sample/mL. An aliquot of 900 µL of cleaned extract was transferred into an autosampler vial and mixed with 50 µl of isotopically labelled sterigmatocystin.

To extract sterigmatocystin from beer, it was first degassed in an ultrasonic bath. Then 10 mL was diluted with 5 mL of phosphate buffer (PBS, pH 7.4) and extracted/cleaned up using a new immunoaffinity column for sterigmatocystin (kindly provided by R-Biopharm-Rhône, Glasgow, UK). After washing the column with 2 mL water, sterigmatocystin was eluted in a graduated glass vial with 6 mL acetonitrile. The extract was concentrated under a gentle flow of nitrogen and brought to 2 mL with acetonitrile-water 40+60 v/v. The amount of matrix equivalent in the extract was 5 g sample/mL. An aliquot of 900 µl of cleaned extract was transferred into an autosampler vial and mixed with 50 µl of isotopically labelled sterigmatocystin.

Fera: to 5 gram dry milled homogenate, 15 µL of isotopically labelled sterigmatocystin in acetonitrile was added (equivalent to 1.5 µg/kg). The sample was extracted with 20 mL of acetonitrile/water (80/20 v/v) using an orbital shaker for 2 hours. For slurried samples the amount of sample used and proportion of acetonitrile/water in the extraction solvent were adjusted depending on the amount of water used in the slurry preparation. After centrifugation, 500 µL of the clear supernatant was transferred into a vial and diluted with 500 µL acetonitrile/water 20/80 v/v. The extract was cooled overnight in a refrigerator and then filtered using a 0.22 µm nylon syringe filter into an autosampler vial. The amount of matrix equivalent in the final extract was 0.125 g sample/mL.

BPI: depending on the slurry composition, 6.25–7.5 g of the sample slurry was extracted with 8.25 mL of acetonitrile using an Ultra Turrax (T25 Basic Ultra Turrax) during 5 minutes. After centrifugation, 675–800 µL of the clear supernatant was transferred into a glass vial, 50 µL of isotopically labelled sterigmatocystin (1.88 ng/mL) in acetonitrile was added, and the volume was made up to 1.0 mL with water. After mixing, the extract was filtered through a disposable PTFE syringe filter (0.45 µm) into an autosampler vial. The amount of matrix equivalent in the final extract depended on the slurry composition and ranged from 0.167 to 0.125 g sample/mL.

3.4. LC-MS/MS analysis

RIKILT: 10 µL of the extract was injected into an UHPLC-MS/MS system. The system consisted of a Waters Acquity LC-system (degasser, pumps, autosampler, column, oven) and a QTRAP 6500 hybrid quadrupole ion trap system from Applied Biosystems. Separation was performed on a HSS T3 column (1.8 µm particle size, 100x2.1 mm, Waters) maintained at 60 °C, with a mobile-phase gradient of water and methanol/water (95/5 v/v) (both containing 1 mM of ammonium formate and 1 % of formic acid) from 90:10 (1 minute) to 0:100 in 3 minutes, then isocratic for 3 minutes. The flow rate was 0.5 mL/minute. For part of this survey, analysis of the extracts were done together with regular multi-mycotoxin analysis for which different chromatographic conditions were used: separation was done on an Ultra Aqueous C18 column (3 µm particle size, 100x2.1 mm, Restek), maintained at 35 °C, with a mobile-phase gradient of water and methanol/water (95/5 v/v) (both containing 1 mM of ammonium formate and 1 % of formic acid) from 100:0 (1 minute) to 0:100 in 8 minutes, then isocratic for 2 minutes, and a flow rate of 0.4 mL/minute.

MS/MS measurement was performed using positive electrospray ionisation applying the following conditions: Curtain gas, 10.0; Collision gas (nitrogen), medium; IonSpray Voltage, 5 000 V; temperature 400 °C; Ion Source Gas 1 and 2, 35 and 40, respectively; entrance potential, 10 V. For sterigmatocystin, using $[M+H]^+$ (m/z 325) as precursor ion, four transitions were measured: m/z 281 (49 V), m/z 310 (35 V) [quantification ion], m/z 253 (61 V) and m/z 282 (39 V). For the isotopic label ($^{13}C_{18}$), 343 m/z was used as precursor ion with m/z 297 (49 V) [quantifier] and m/z 327 (35 V) as product ions. All transitions were measured in one event using dwell times of 50 ms.

Analyst software version 1.5 (AB Sciex) was used for data-evaluation. Peak assignment and integration were manually verified by the operator. Quantification was based on multi-level calibration using solvent standards of sterigmatocystin (concentrations corresponding to 0.25, 0.50, 1.5, 5.0 and 10 µg/kg in the sample, internal standard at 1.5 µg/kg) which were injected prior and after the extracts. Responses in extracts and standards were normalized to the internal standard. Since the internal standard was added after extraction, it corrected for matrix effects only, not for recovery. In case of positive samples, the concentration found was corrected for the average recovery calculated from all QC samples analysed during the survey (see Section 3.6).

UCSC: 5 µL of the extract was injected into an HPLC-MS/MS system consisting of a LC 1.4 Surveyor pump, a Quantum Discovery Max triple-quadrupole mass spectrometer (Thermo-Fisher Scientific, San Jose, CA, USA) and a PAL 1.3.1 sampling system (CTC Analytics AG, Zwingen, Switzerland). The system was controlled by Xcalibur 1.4 software (Thermo-Fisher). Sterigmatocystin was separated on a Betasil RP-18 column (5 µm particle size, 150x2.1 mm, Thermo-Fisher) with a mobile-phase gradient of acetonitrile and water (both acidified with 0.2 % formic acid) from 40:60 to 75:25 in 3 minutes, then isocratic for 8 minutes; the flow rate was 0.2 mL/minute.

MS/MS measurement was performed using positive atmospheric pressure chemical ionisation (APCI) applying the following conditions: voltage 4.0 kV, sheath and auxiliary gas 29 and 5 psi, respectively, temperature of the heated capillary 270 °C. Argon was used as collision gas; the pressure was set to 1.5 mTorr. For sterigmatocystin, using $[M+H]^+$ (m/z 325) as precursor ion, three transitions were measured: 310 (24 V) [quantifier], 281 and 253 m/z (35 V). For the isotopic label ($^{13}C_{18}$), m/z 343 was used as precursor ion with m/z 327 (24 V) [quantifier], 297 and 268 (35 V) as product ions.

Quantitative determination was performed using ICIS algorithm (LC-Quan 2.0 software). For LC-MS calibration, five standard solutions (between 0.66 and 1.33 µg/L) were injected. For quantitative determination, linear calibration (equal weighting, ignore origin) was performed. Since the internal

standard was added after extraction and clean up, it corrected for matrix effects only, not for recovery. In case of positive samples, the concentration found was corrected for the average recovery calculated from all QC samples analysed during the survey (see Section 3.6).

Fera: 2 µL of the extract was injected into an UPLC-MS/MS system. The system consisted of a Waters Acquity LC-system (degasser, pumps, autosampler, column, oven) and a TQ-S triple quadrupole MS/MS system from Waters. Separation was performed on a HSS T3 column (1.8 µm particle size, 100x2.1 mm, Waters) maintained at 40 °C, with a mobile-phase gradient of 1 mM ammonium formate in water and methanol/acetonitrile (50/50 v/v) from 95:5 (0.2 minutes) to 5:95 in 3 minutes, then isocratic for 3.6 minutes. The flow rate was 0.4 mL/minute.

MS/MS measurement was performed using positive electrospray ionisation applying the following conditions: capillary voltage, 3 kV; desolvation temperature, 500 °C; desolvation gas flow rate, 1 000 L/h; nebuliser gas flow, 7 bar; source temperature, 150 °C; cone gas flow rate, 100 L/h; cone voltage, 40 V. For sterigmatocystin, using $[M+H]^+$ (m/z 325) as precursor ion, two transitions were measured: m/z 310 (23 V) [quantification ion], and m/z 281 (35 V). For the isotopic label ($^{13}C_{18}$), 343 m/z was used as precursor ion with m/z 297 (36 V) as product ion.

Masslynx software (Waters) was used for data-evaluation. Peak assignment and integration were manually verified by the operator. Quantification was based on multi-level calibration using solvent standards of sterigmatocystin (concentrations corresponding to 0.20, 0.50, 1.5, 2.5, 5.0 and 10 µg/kg in the sample, internal standard at 1.5 µg/kg) included in the sequence. Responses in extracts and standards were normalized to the internal standard. Since the internal standard was added to the sample before extraction, it corrected for both recovery and matrix effects. Hence in this case, for positive samples, the concentration found was inherently corrected for the recovery.

BPI: 20 µL of the extract was injected into an HPLC-MS/MS system. An injection program was used in which the sample was diluted in water with a ratio of 20:80 before being introduced onto the analytical column. The system consisted of an Agilent Series 1200 liquid chromatograph with a degasser (G1379B), autosampler (Hip/ALS G1367A) with thermostat (FC/ALS Therm G1330B), binary pump (G1312A) and a thermostatic column department (TCC G1316A). This HPLC was coupled to an Agilent 6410 triple quadrupole mass spectrometer. Separation was performed on a Zorbax Eclipse XDB C18 column (3.5 µm particle size, 150x2.1mm) maintained at 30 °C, with a mobile-phase gradient of water and methanol (both containing 5 mM of ammonium formate and 0.1 % of formic acid) from 70:30 (1 minute) to 0:100 in 10 minutes, then isocratic for 5 minutes. The flow rate was 0.3 mL/minute. In the course of the survey the column was replaced by an ACE C18 column (3.5 µm particle size, 150 mm x 2.1 mm).

The MS/MS measurement was performed using positive electrospray ionisation applying the following conditions: drying gas temperature, 300 °C; drying and nebulizing nitrogen gas 11 L/minutes and 40 psi, respectively; fragmentor voltage, 135 V; collision gas (nitrogen), 1.5mTorr. For sterigmatocystin, using $[M+H]^+$ (m/z 325) as precursor ion, two transitions were measured: m/z 310 (30 V) [quantification ion], and m/z 281 (30 V). For the isotopic label ($^{13}C_{18}$), m/z 343 was used as precursor ion with m/z 327 (30 V) [quantifier] and m/z 297 (30 V) as product ions. All transitions were measured in one event using dwell times of 50 ms.

For instrument control, Agilent Mass Hunter data acquisition Triple Quad B.01.04 and for data processing Agilent MassHunter Workstation Qualitative Analysis B.01.04 was used. Peak assignment and integration were manually verified by the operator. Quantification was based on multi-level calibration using solvent standards of sterigmatocystin (concentrations 0.016, 0.031, 0.094, 0.31, 0.65,

0.94 µg/L in the extract, internal standard at 0.094 µg/L) included in the sequence. Responses in extracts and standards were normalized to the internal standard. Since the internal standard was added after extraction, it corrected for matrix effects only, not for recovery. In case of positive samples, the concentration found was corrected for the average recovery calculated from all QC samples analysed during the survey (see Section 3.6).

3.5. Validation

3.5.1. Validation approach

To demonstrate the fitness-for-purpose of the analysis methods to be used for this survey at the four laboratories, a (re-)validation was performed. Due to the lack of specific regulations or guidance documents for the validation of mycotoxins in products of plant origin, SANCO/12571/2013 (applying to pesticides in food/feed) was used as guidance document for validation and on-going analytical quality control. In agreement with the terms of reference, for average recovery and precision, the criteria as specified in Commission Regulation (EC) No 401/2006 for aflatoxin B_1 were applicable.

Based on the experience with the determination of sterigmatocystin in cereals and derived products, it was known that for different type of cereals or cereal products, the sample preparation has only a minor effect on the recovery. Since matrix-dependent ion suppression effects in LC-MS/MS analysis are compensated for by the use of an isotopically labelled standard, minor differences are expected between the method performance for the various cereals and cereals products. Therefore it was considered justified to consider cereal grains as one matrix and to validate them as one commodity group. For this group, the validation was performed by spiking each of the six types of cereal grains to be included in the survey in duplicate at two levels (0.5 µg/kg and 5 µg/kg), i.e. a validation set consisting of 12 samples per level was obtained. A similar approach was followed for the cereal products. Six cereal products from different food categories (pasta, breakfast cereals/muesli, bread, biscuit, pastry/cake and cereal-based infant food) were used for this purpose. Nuts and beer were considered as two separate matrix groups. For these groups the validation set was limited to triplicates of peanut and hazelnut and duplicates of three different beer varieties, again spiked at two levels (0.5 µg/kg and 5 µg/kg). All matrices included in the validation were also analysed without spiking, as well as a reagent blank. The linearity of the LC-MS/MS measurement was established through five calibration standards in solvent, covering the relevant concentration range, to which the labelled internal standard was added at a fixed concentration. From these initial in-house validations, the linearity, recovery, repeatability, selectivity, LOQ, and LOD were derived. In addition, the stability of retention time and ion ratios in solvent standards and extracts were determined.

3.5.2. Validation criteria

Linearity of normalized response vs concentration: the back-calculated concentrations, using the equation of the calibration curve, should not deviate more than 20 %.

Recovery: the recommended value for average recovery is 50–120 % at the 0.5 µg/kg level and 70–110 % at the 5 µg/kg level (derived from Commission Regulation (EC) No 401/2006 for aflatoxin B_1).

Precision: the recommended repeatability (RSD_r) is based on Horwitz and should not exceed 33 % at the 0.5 µg/kg level and 23 % at the 5 µg/kg level (derived from Commission Regulation (EC) No 401/2006 for aflatoxin B_1).

Selectivity: the response for sterigmatocystin in the non-fortified samples should not exceed 30 % of the response at the lowest fortification level.

LOQ: here the LOQ is defined as the lowest level for which it has been demonstrated that the criteria for average recovery and repeatability are met. The target LOQ for this study was 0.5 µg/kg, which based on experience was expected to be achievable by all four laboratories under routine conditions. Since 0.5 µg/kg was the lowest investigated level in this study, by definition the LOQ will not be lower than this value. For this survey, the fit-for-purpose LOQ should not exceed 1.5 µg/kg (EFSA CONTAM Panel, 2013).

LOD: the LOD is defined here as the level corresponding to a signal-to-noise ratio (S/N) of three. Here noise is 'peak-to-peak noise' as manually determined from extracted ion chromatograms of the 0.5 µg/kg fortifications. The response should be taken for the transition with the lowest S/N (i.e. the qualifier ion).

Matrix effects: since matrix effects are compensated for through the use of the isotopically labelled internal standard, they were not evaluated in detail and no criteria were applicable.

Variability of retention time and ion ratios: to gain insight in the variability of retention times and the ion ratio of at least two diagnostic ions of sterigmatocystin, the individual retention time and ion ratio deviations relative to the average of solvent standards were calculated. The retention time of sterigmatocystin in the samples should not deviate more than 0.05 minutes from the ^{13}C-isotope in the same solution, and the ion ratio deviations not more than ± 30 %.

3.5.3. Additional quality control

Interlaboratory comparability: for sterigmatocystin, no proficiency test existed at the time of this study. Therefore the comparability of results between the four laboratories performing the analyses was verified by exchange of solvent standards used for spiking and calibration, and by analysis of a sample containing sterigmatocystin. Solvent standards containing sterigmatocystin at 50–100 ng/mL in acetonitrile were exchanged between the four laboratories. The sample containing sterigmatocystin was a composite sample prepared by RIKILT from three dry-milled samples of 'broken rice' that tested positive (approx. 2 µg/kg) in the national feed monitoring program in the Netherlands in 2013. After thoroughly mixing the dry rice powder (< 1mm), portions of the naturally contaminated material were distributed among the partner laboratories.

On-going analytical quality control: with each batch of survey samples, one or more spiked samples were included in the batch. These included samples for which no previous recovery experiments had been done (typically a random sample from the batch). This way, method performance in time and extension of applicability to other matrices from the same product group were assessed. These data were also used to establish the long-term within-laboratory reproducibility and for estimation of measurement uncertainty.

Storage stability: the stability of standards was assessed by comparison of newly purchased stock solutions against the ones already available in the laboratory, and by comparison of freshly prepared working solutions from the existing stock against older working solutions.

The storage stability of sterigmatocystin in homogenised samples was assessed for slurried and dry milled samples both stored at < -18 °C. For this purpose portions of a '1 wheat+1.5 water' slurry were spiked at 1.5 µg/kg and stored in the freezer together with non-spiked portions. At several time intervals, a blank and a spiked sample were taken from the freezer. The blank was freshly fortified and then both samples were analysed and the recovery determined. For the dry milled sample an incurred rice (feed) material was used containing approximately 2 µg/kg.

Identification of sterigmatocystin: identification of sterigmatocystin was based on retention time and the presence of coinciding peaks for at least two diagnostic transitions in the correct abundance ratio. In general the retention time of sterigmatocystin in samples should not deviate more than 0.20 minutes from the reference retention time in solvent standards. Since an isotopically labelled standard is used in this study, a more stringent requirement was set, i.e. the retention time of sterigmatocystin should not differ more than 0.05 minutes from its isotopic label. The ion ratio of the two diagnostic ions (least abundant/most abundant) of sterigmatocystin in the samples should be consistent with that obtained during validation and in any case should not deviate more than 30 %.

3.6. Reporting of results

The results obtained for the survey samples were only considered valid when the linearity and recoveries obtained for a particular batch complied with the criteria mentioned in Section 3.5.2, when the criteria for identification mentioned in Section 3.5.3 were met, and when in a known (reagent) blank no sterigmatocystin was identified.

The results obtained were corrected for recovery, except when the internal standard was added to the sample before extraction. In the latter case recovery correction was inherent to the procedure. For recovery correction for cereals and cereal-products, each laboratory used the average recovery value calculated from all fortified samples analysed concurrently with their survey samples. These average recoveries were 105 %, 87 % and 93 % for RIKILT, BPI and UCSC, respectively.

Taking the EFSA reporting format into account, there were three possible types of analysis result:

i) 'Numerical Value': interpreted here as samples in which sterigmatocystin was found at levels equal to or higher than the lowest validated level (≥ 0.5 µg/kg).

ii) 'Value below the lower limit of the working range', interpreted here as samples in which sterigmatocystin was identified but below the lowest validated level (< 0.5 µg/kg).

iii) 'Non Detected Value (<LOD)', interpreted here as samples in which no sterigmatocystin was identified.

In the EFSA database, values below the lower limit of the working range (< 0.5 µg/kg) are included as 'Comment to the result'. The value provided here should be regarded as indicative because no data on recovery and precision were generated below the 0.5 µg/kg level, and the response may be below the lowest calibration level. For LOD a value of 0.1 µg/kg was entered in the EFSA database which was the estimated average obtained for the different cereal and nut samples by the laboratories. For beer this was 0.01 µg/kg.

For the average and median concentrations of sterigmatocystin in the samples, and the 75th and 95th percentiles, three values were calculated, the lower bound (LB), middle bound (MB) and upper bound (UB). For the lower bound values, zero was used for '< LOD', and the average LOD (0.1 µg/kg) was used in case sterigmatocystin was identified but below the LOQ. For the middle bound values 0.05 µg/kg (half the average LOD) was used for '< LOD', and 0.25 µg/kg (half the LOQ) in case sterigmatocystin was identified but below the LOQ. For the upper bound values, the average LOD (0.1 µg/kg) was used for '<LOD', and the LOQ (0.5 µg/kg) was used in case sterigmatocystin was identified but below the LOQ.

RESULTS

4. Method validation

4.1. Initial validation

The (adjusted) method for the determination of sterigmatocystin in the different cereals and processed cereal products was validated at 0.5 µg/kg and 5 µg/kg by each of the analytical laboratories. The same method was validated for nuts by one laboratory assigned for analysis of all nut samples. Another method dedicated for beer was validated by the laboratory performing the beer analysis. The samples used for validation were obtained by each laboratory themselves so different samples of the same product were used in the different validation exercises.

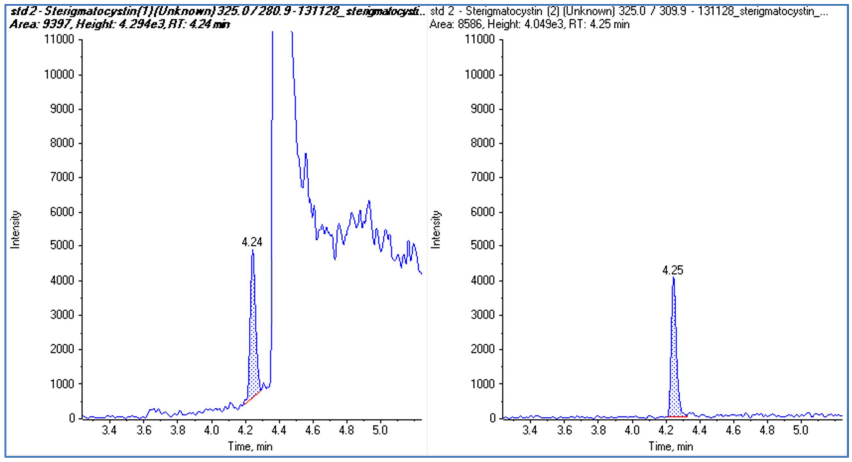
For all four laboratories, the linearity requirements were met in the range equivalent to 0.2–10 µg/kg. In blank samples no sterigmatocystin was identified. The one exception to this was an oats sample included in the initial validation by Fera. A confirmatory analysis revealed that the sample used for validation was positive for sterigmatocystin. For this reason another oats sample was taken and the analysis repeated. For all four laboratories (MS/MS instruments), transition m/z 325 → m/z 310 was slightly lower in abundance than m/z 325 → 281 but in many cases it provided a better signal-to-noise ratio in the samples and therefore it was used as default for quantification. Examples of extracted ion chromatograms for calibration standards at a level corresponding to approximately half the lowest validation level, blank samples, and samples spiked at 0.5 µg/kg are shown in Figures 1–4.

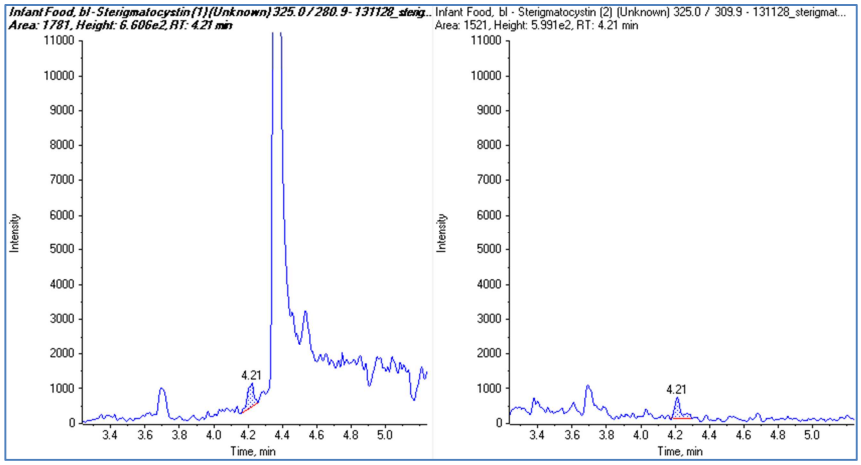
The matrix effects determined for the different matrices by comparison of the absolute response obtained in spiked samples with the corresponding solvent-based standards were not very pronounced, most likely due to the fact that sample preparation involved either an 8–16x dilution or a clean-up. In most cases, ion suppression was less than 30 %. Matrix effects were not quantified in further detail because they were irrelevant for calibration due to the use of the isotopically labelled internal standard, and also because the adverse effect on the LOD was minor.

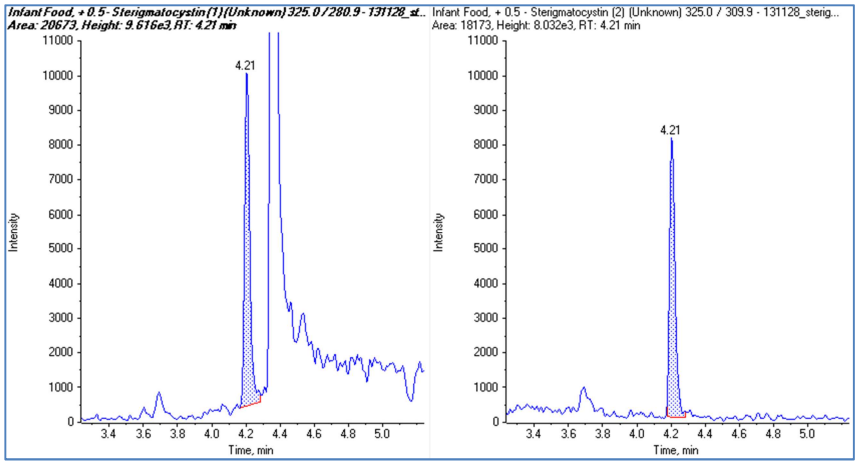
An overview of the individual recoveries of all samples analysed during initial validation by the four laboratories, and the average recoveries and repeatabilities (RSD_r) for each product group are given in Tables 2–4. The average recoveries and repeatabilities all met the requirements from which it was concluded that the methods used by the different laboratories were fit-for-purpose for quantitative analysis of sterigmatocystin in the investigated product (groups) down to 0.5 µg/kg.

The LOD depends on the signal-to-noise ratio observed in the extracted ion chromatograms. Since it is affected by the matrix and the condition of the LC-MS/MS system at the time of analysis, it is considered less meaningful to give an exact fixed value. Therefore, a range is given here, based on the observations during validation and the analysis of the survey samples: 0.05–0.15 µg/kg for cereal grains, processed cereals products, and nuts, and 0.005–0.01 µg/kg for beer.

The retention times within the validation sequences were very consistent. Deviations of the absolute retention time of sterigmatocystin relative to the reference value (average of solvent standards injected in the same sequence) were less than 0.05 minutes. Deviations of individual ion ratios relative to the reference were typically 10 %, with maximum deviations up to 18 % for some of the 0.5 µg/kg spikes.

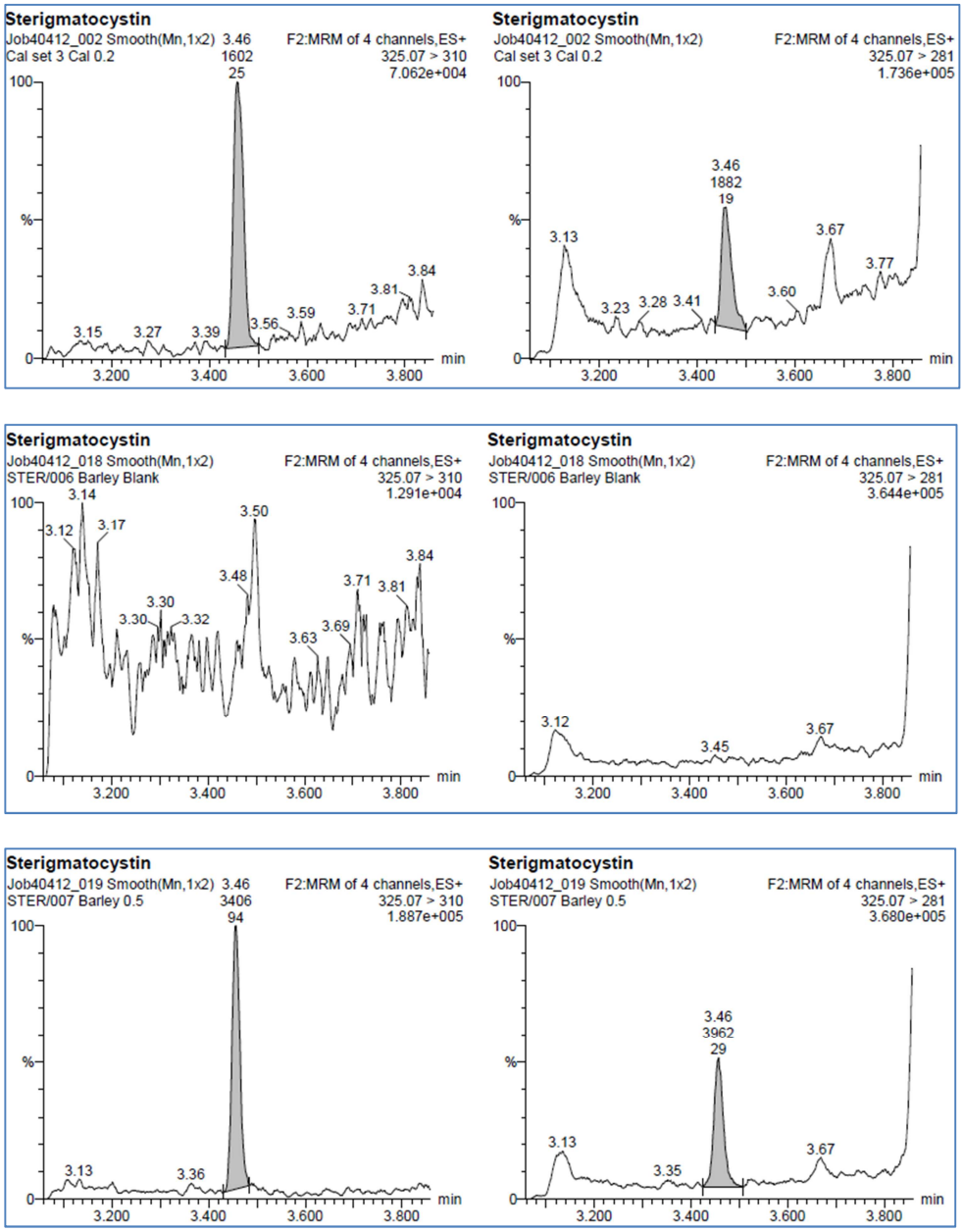






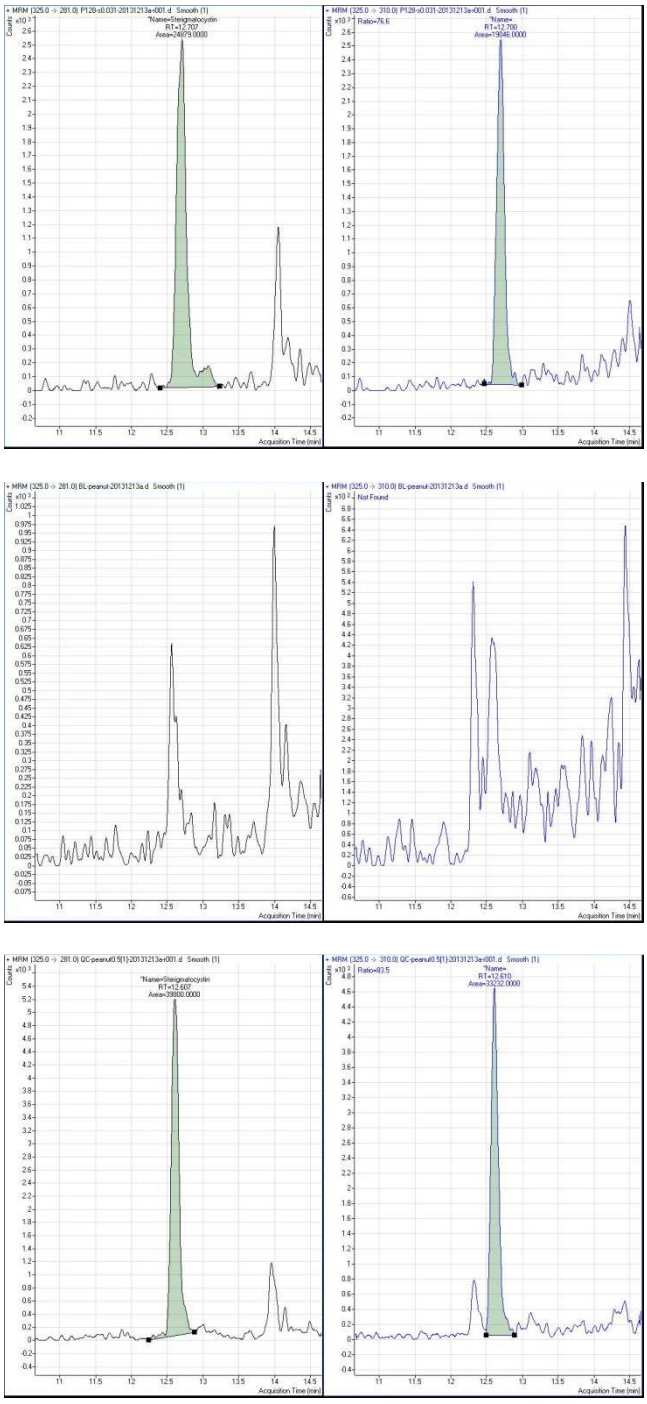
Left: transition m/z 325 → 281, right m/z 325 → m/z 310. Calibration standard in solvent 0.031 ng/mL (corresponding to 0.25 µg/kg in cereal) (top), non-fortified cereal-based infant food (middle) and fortified at 0.5 µg/kg (RIKILT).

Figure 1: Example chromatograms for sterigmatocystin obtained during validation (baby food)



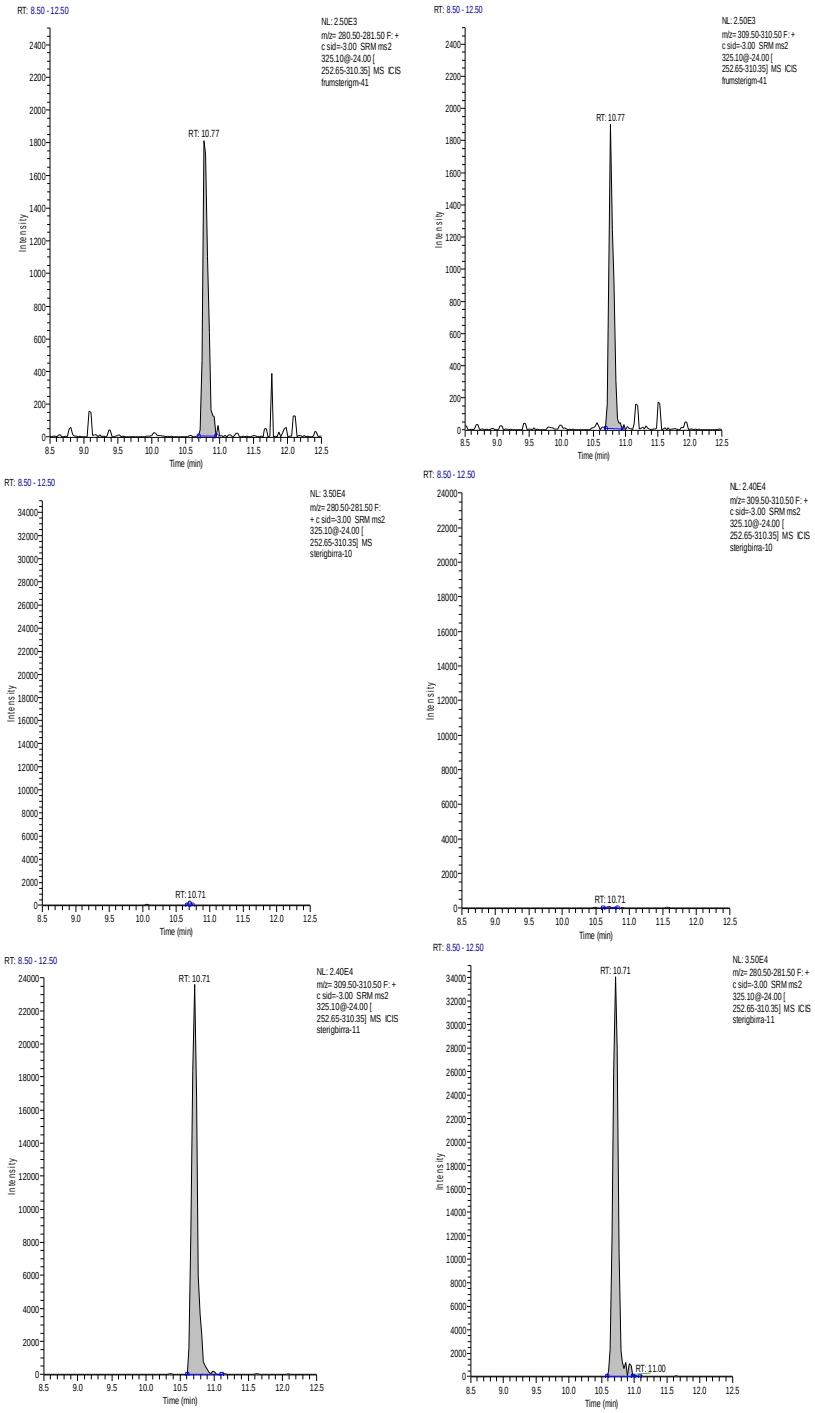
Left: m/z 325 → m/z 310, right: transition m/z 325 → 281. Calibration standard in solvent 0.025 ng/mL (corresponding to 0.20 µg/kg in cereal) (top), non-fortified barley (middle) and fortified at 0.5 µg/kg (Fera - Food and Environment Research Agency, UK).

Figure 2: Example chromatograms for sterigmatocystin obtained during validation (barley)



Left: transition m/z 325 → 281, right m/z 325 → m/z 310. Calibration standard in solvent 0.031 ng/mL (corresponding to 0.25 µg/kg peanut) (top), non-fortified peanut (middle) and fortified at 0.5 µg/kg (BPI - Benaki Phytopathological Institute, Greece).

Figure 3: Example chromatograms for sterigmatocystin obtained during validation (peanut)



Left: transition m/z 325 → 281, right m/z 325 → m/z 310. Calibration standard in solvent 1.25 ng/mL (corresponding to 0.25 µg/kg beer) (top), non-fortified beer (middle) and fortified at 0.5 µg/kg (Università Cattolica del Sacro Cuore – UCSC, Italy).

Figure 4: Example chromatograms for sterigmatocystin obtained during validation (beer)

Table 2: Recoveries obtained for sterigmatocystin in cereals

Product group 1: cereal grains					
Matrix	**Spike (µg/kg)**	**RIKILT**	**UCSC**	**Fera**	**BPI**
		Recovery (%)			
wheat	0.5	100	104	109	84
wheat	0.5	99	92	114	84
wheat	5	95	93	105	84
wheat	5	103	92	97	88
barley	0.5	93	104	111	80
barley	0.5	105	99	107	93
barley	5	99	96	102	82
barley	5	96	93	88	90
oats	0.5	78	96	74	91
oats	0.5	70	102	79	100
oats	5	102	103	71	102
oats	5	98	97	70	112
maize	0.5	96	89	114	84
maize	0.5	95	92	110	98
maize	5	90	91	107	102
maize	5	88	92	105	91
rye	0.5	102	101	122	108
rye	0.5	101	102	109	100
rye	5	95	103	97	89
rye	5	96	101	113	106
rice	0.5	102	94	109	94
rice	0.5	94	99	124	114
rice	5	90	96	101	74
rice	5	89	92	100	71
Average	**0.5**	**95**	**98**	**107**	**94**
RSD (%)	**0.5**	**11**	**5**	**14**	**11**
Average	**5**	**95**	**96**	**96**	**91**
RSD (%)	**5**	**5**	**4**	**14**	**14**

RSD: relative standard deviation.

BPI: Benaki Phytopathological Institute, Greece; Fera: Food and Environment Research Institute, UK; UCSC: Università Cattolica del Sacro Cuore, Italy.

Table 3: Recoveries obtained for sterigmatocystin in processed cereal products

Product group 2: processed cereal products					
Matrix	**Spike (µg/kg)**	**RIKILT**	**UCSC**	**Fera**	**BPI**
		Recovery (%)			
pasta (spaghetti)	0.5	99	90	110	80
pasta (spaghetti)	0.5	93	90	119	92
pasta (spaghetti)	5	96	89	94	82
pasta (spaghetti)	5	92	92	103	80
breakfast cereal	0.5	111	103	119	100
breakfast cereal	0.5	98	100	111	100
breakfast cereal	5	96	89	100	93
breakfast cereal	5	99	92	104	102
bread	0.5	100	102	115	84
bread	0.5	110	103	87	79
bread	5	106	91	112	89
bread	5	99	90	78	89
biscuit	0.5	102	90	94	106
biscuit	0.5	96	89	88	95
biscuit	5	97	90	93	94
biscuit	5	98	95	100	116
cake/pastry	0.5	99	106	109	98
cake/pastry	0.5	97	100	104	110
cake/pastry	5	93	88	101	102
cake/pastry	5	98	89	107	101
infant food	0.5	100	90	103	108
infant food	0.5	99	90	104	112
infant food	5	97	90	98	85
infant food	5	99	90	96	93
Average	**0.5**	**100**	**96**	**105**	**97**
RSD (%)	**0.5**	**5**	**7**	**10**	**12**
Average	**5**	**97**	**90**	**99**	**94**
RSD (%)	**5**	**4**	**2**	**9**	**11**

RSD: relative standard deviation.

BPI: Benaki Phytopathological Institute, Greece; Fera: Food and Environment Research Institute, UK; UCSC: Università Cattolica del Sacro Cuore, Italy.

Table 4: Recoveries obtained for sterigmatocystin in beer and nuts

Beer (UCSC – Università Cattolica del Sacro Cuore)

Matrix	Spike (µg/kg)	Recovery (%)
beer-1	0.5	92
beer-1	0.5	90
beer-1	5	91
beer-1	5	93
beer-2	0.5	90
beer-2	0.5	93
beer-2	5	90
beer-2	5	89
beer-3	0.5	91
beer-3	0.5	88
beer-3	5	89
beer-3	5	92
Average	**0.5**	**91**
RSD (%)	**0.5**	**2**
Average	**5**	**90**
RSD (%)	**5**	**2**

Nuts (BPI – Benaki Phytopathological Institute, Greece)

Matrix	Spike (µg/kg)	Recovery (%)
hazelnuts	0.5	87
hazelnuts	0.5	79
hazelnuts	0.5	89
hazelnuts	5	89
hazelnuts	5	113
hazelnuts	5	113
peanut	0.5	98
peanut	0.5	106
peanut	0.5	105
peanut	5	114
peanut	5	94
peanut	5	108
Average	**0.5**	**94**
RSD (%)	**0.5**	**11**
Average	**5**	**105**
RSD (%)	**5**	**10**

RSD: relative standard deviation.

4.2. Method from this survey *vs* methods from recent literature

One of the requirements stated in the terms of reference was that the method used for this survey should be state-of-the-art. To verify this, the methods used here were compared with methods published in the scientific literature from the past two years. Virtually all recent methods were using similar extraction approaches followed by LC-MS/MS analysis, as was done in this study. Methods were reported for cereals (Alkadri et al., 2014; Arroyo-Manzanares et al., 2014; Malachova et al., 2014) and for nuts (Arroyo-Manzanares et al., 2013; Varga et al., 2013). For beer a method was reported by Sasaki et al. (2014) using an immunoaffinity cleanup as in this study, but followed by LC-single stage MS instead of MS/MS. In terms of recoveries and precision, the performance was similar to that obtained in the current validation. The reported LODs were 0.2–1 µg/kg (here: 0.05–0.15 µg/kg) and LOQs 0.3–3 µg/kg (here 0.5 µg/kg). However, a direct comparison is difficult due to the different definitions and ways of calculation. Therefore a comparison was made of the lowest levels that were actually validated. The lowest validated levels in the cited papers varied from 1 to 5 µg/kg, compared to 0.5 µg/kg in this study. From this it was concluded that the method used for the survey was state-of-the-art with similar or better sensitivity as published in the recent literature.

4.3. Interlaboratory comparison

To ensure comparability of results obtained by the four laboratories, solvent standards used for spiking and calibration were exchanged and a sample of rice with incurred sterigmatocystin was distributed.

For comparison of the sterigmatocystin standards the solutions supplied were diluted by the laboratory to achieve a concentration within the calibration range. The concentration was then determined and compared with the theoretical one. If a solution with a given concentration was supplied, it was diluted to the same concentration of one of the calibration standards. The two standards were alternatingly injected in at least triplicate and the differences between the absolute average responses of each standard were calculated. The solvent standards used by RIKILT, Fera, UCSC and BPI differed 9 % or less.

The sample of naturally contaminated rice (feed material from 2013) was prepared by RIKILT and first measured in December 2013. The amount of material available was insufficient to perform a full homogeneity study. Portions of the dry milled sample material were sent to the partner laboratories in the first week of February 2014 and subsequently analysed. In Table 5 the individual analysis results for the different replicates are given. The results were in good agreement with each other, indicating that the four laboratories produced comparable and consistent results.

Table 5: Analysis results for a naturally contaminated rice sample analysed by four laboratories

Replicate	Measured concentration in µg/kg			
	RIKILT	**UCSC**	**Fera[(a)]**	**BPI**
1	2.05	1.9	1.81	2.16
2	2.39	2.1	1.64	2.31
3	2.24	2.2		2.16
4	2.01			2.02
5	1.85			
Average	**2.11**	**2.07**	**1.73**	**2.16**
RSD (%)	**10**	**7.4**	**7.0**	**5.4**

RSD: relative standard deviation.

BPI: Benaki Phytopathological Institute, Greece; Fera: Food and Environment Research Institute, UK; UCSC: Università Cattolica del Sacro Cuore, Italy.

(a): the sample was also included as quality control sample with every analysis batch - see Table 6.

4.4. On-going analytical quality control and measurement uncertainty

As part of the batch quality control, one or more samples fortified at 1.5 µg/kg were included with each batch of samples analysed. Fera also included the incurred rice (feed) sample with every batch. The individual recoveries for each of the four laboratories are given in Appendix 2. Most of the individual recoveries were within 70–110 %. In some cases higher recoveries were obtained, however, the average recoveries were always within the recommended range. In Table 6, for each of the food commodities, i.e. cereal grains, processed cereal products, beer and nuts, the average recovery and the reproducibility (RSD_R) obtained by each laboratory are summarized. The RSD_R ranged from 2 to 18 % which was well within the recommended value from Commission Regulation (EC) No 401/2006 for aflatoxin B_1 (Horwitz equation at 1.5 µg/kg = 43 %).

The freezer storage stability of sterigmatocystin in slurry (wheat) was evaluated over a period of 10 months. In that time no significant degradation occurred. A milled rice sample (feed) with incurred sterigmatocystin was analysed 10 times in the period February-November. No degradation was observed in this period, from which it was concluded that sterigmatocystin is stable for at least 10 months in both wheat slurry and dry milled rice stored in the freezer.

The measurement uncertainty of RIKILT, BPI and UCSC was estimated using the within-laboratory reproducibility (RSD_R) (Table 6) combined with the variability from sample inhomogeneity (RSDs from Table 5). The combined variability was calculated as the square root of the sum of the squares of the two RSD values. For Fera, the measurement uncertainty was estimated by the reproducibility of the measurement of the incurred rice sample that was analysed with every batch. This also covered the contribution from sample inhomogeneity. The expanded measurement uncertainty was the combined variability multiplied by a coverage factor (k) of 2, and was in the range of 19–40 %.

Table 6: Average recovery and reproducibility of quality control samples

	Number	Average (%)	RSD_R (%)	Exp. MU (%)
RIKILT				
Cereal grains	25	103	7	
Cereal products	19	107	8	
Cereal grains/products	44	105	8	26
Fera				
Cereal grains	21	99	14	
Cereal products	18	101	14	
Cereal grains/products	39	100	14	
Incurred rice (feed)	9	2.07[(a)]	20	40
BPI				
Cereal grains	17	86	15	
Cereal products	24	87	18	
Cereal grains/products	43	87	16	34
Nuts	5	81	10	23
UCSC				
Cereal grains	13	93	1.6	
Cereal products	8	93	1.2	
Cereal grains/products	21	93	1.5	19
Beer	2	92	-	-

Exp.MU: expanded relative measurement uncertainty; RSDR: Relative standard deviation under reproducibility conditions.

BPI: Benaki Phytopathological Institute, Greece; Fera: Food and Environment Research Institute, UK; UCSC: Università Cattolica del Sacro Cuore, Italy.

(a): average concentration in incurred quality control sample in µg/kg.

5. Samples collected

In total 1 259 samples were collected for this survey and analysed, which was substantially above the target as set in the sampling plan (Table 7). Only for rye grain and maize grain the number of samples was below the anticipated number. However, this was compensated for by taking samples of those products with minimal processing (wholemeal flour). Unprocessed rice grain was not readily available except when grown in Europe (Italy, Greece), which was the reason for a lower number of samples than foreseen in this category. This was also compensated for by additional (wholegrain) rice samples.

In the survey organic products were included, not with the aim to investigate differences between potential contamination of organic and non-organic products, but to reflect consumer purchase behaviour. Approximately 5 % of organic samples were foreseen. For most products this was achieved with barley and durum wheat grain, bread and fine bakery ware, and beer as exceptions.

Table 7: Overview of samples types collected and analysed

Product	Target number	Samples analysed	% realised	Organic samples	% organic
OVERALL	**1 000**	**1 259**	**126 %**	**108**	**8.6 %**
ALL CEREALS (except beer)					
CEREAL GRAINS	**400**	**429**	**107 %**	**36**	**8.4 %**
Wheat (soft)	160	169	106 %	16	9.5 %
Wheat (hard/durum)	40	52	130 %	1	1.9 %
Rye	40	35	88 %	5	14.3 %
Maize	40	33	83 %	2	6.1 %
Rice	40	28	70 %	4	14.3 %
Barley	40	59	148 %	1	1.7 %
Oats	40	51	128 %	7	13.7 %
Spelt	-	2	-	1	-
CEREAL PRODUCTS	**500**	**713**	**143 %**		
Products (to be processed)					
Grain milling products	60	125	208 %	21	16.8 %
- *Wheat*		*85*		*8*	
- *Rye*		*11*		*6*	
- *Maize*		*18*		*4*	
- *Rice*		*2*		*1*	
- *Barley*		*1*		*0*	
- *Oats*		*6*		*1*	
- *Other*		*2*		*1*	
Rice	40	89	223 %	11	12.4 %
Pasta	100	115	115 %	10	8.7 %
Products (consumed as such)					
Bread/rolls	150	143	95 %	1	0.7 %
Breakfast cereals (incl. muesli)	50	97	194 %	8	8.2 %
Fine bakery ware	60	90	150 %	1	1.1 %
Cereal-based infant food	40	54	135 %	14	25.9 %
BEER	**50**	**53**	**106 %**	**0**	**0 %**
NUTS	**50**	**64**	**128 %**		
Peanuts	25	28	112 %	3	10.7 %
Hazelnuts	25	36	144 %	2	5.6 %

Sample collection covered a period of more than one year, with emphasis on 2014 (see Figure 5). Samples taken in 2013 were mostly originating from other existing mycotoxin monitoring and control programs. Up to August 2014, the cereal grains and milling products originated from crops grown in 2013. From August 2014 onwards, also samples from the 2014 harvest were included. Although often not indicated on the package, it can be assumed based on the time of sampling that the ingredients of the processed cereal products (pasta, bread, breakfast cereals, cereal-based infant food, etc.) most likely originated from cereals grown in 2013 and maybe before that for products with a long shelf life. Peanuts and hazelnuts were mostly from 2013 harvests.

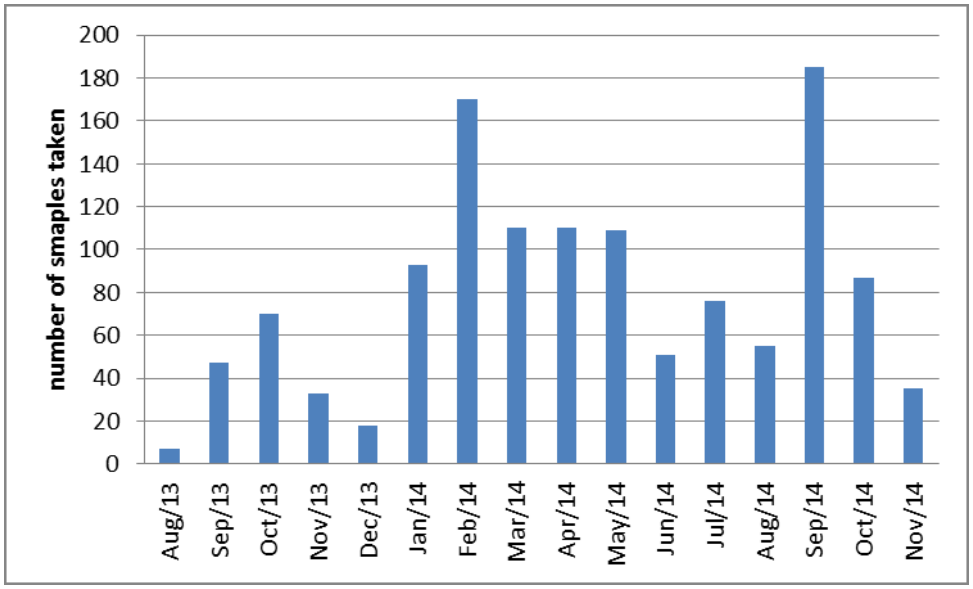


Figure 5: Overview of time of sampling of the products analysed in this survey

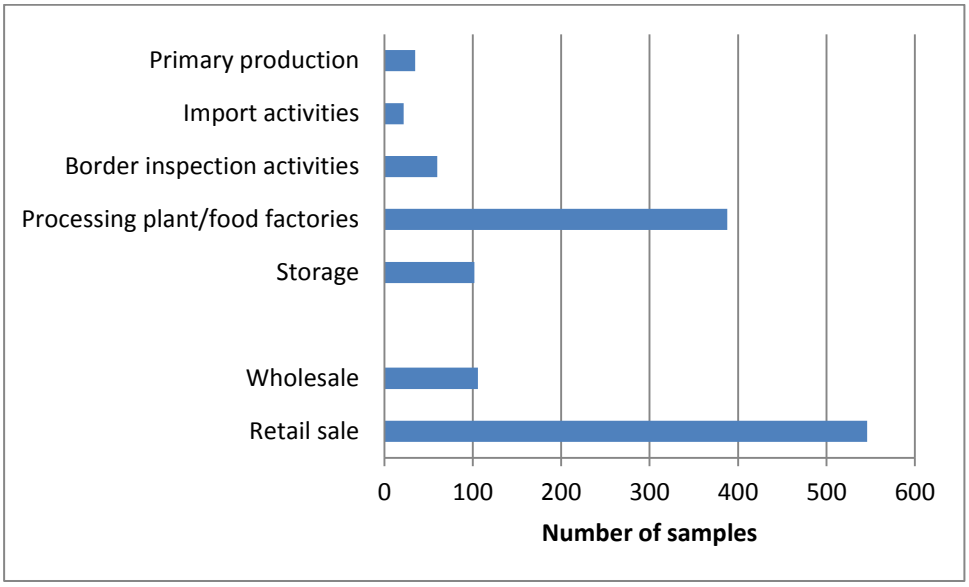


Figure 6: Overview of number of samples taken at various sampling points

Samples were taken at various sampling points (Figure 6). Cereal grains were mainly taken at mills and storage facilities. Import activities and border inspection controls applied mostly to peanuts and hazelnuts and cereals from outside the EU. Cereal products and beer were mostly obtained from wholesale and retail.

The samples were taken in north/west Europe (UK, 276; the Netherlands, 269; Germany, 16), south Europe (Italy 316), south/east Europe (Greece, 275; Cyprus, 22) and eastern Europe (Poland 40, Baltic states, 21). However, products originated from many more countries in and outside Europe and, therefore, the survey is considered to adequately cover the food supply in the EU in 2014. An overview of countries of origin of the various products is provided in Table 8.

Table 8: Overview of sample numbers by countries of origin

		Cereal grains								Cereals products to be cooked			Cereals products direct consumption				Nuts		
	Total	Wheat, soft	**Wheat, durum**	Rye	Maize	Barley	Oats	Spelt	Rice (crop)	Rice	Pasta	Grain millings	Bread & rolls	Infant food	Fine bakery	Breakfast cereals	Peanut	Hazelnut	Beer
	1 259	**169**	**52**	**35**	**33**	**59**	**51**	**2**	**28**	**89**	**115**	**125**	**143**	**54**	**90**	**97**	**28**	**36**	**53**
Europe																			
Austria	1	1																	
Belgium	8										1	3			1	1			2
Bulgaria	1									1									
Croatia	5													3					2
Czech Rep.	1						1												
Cyprus	19									2	3	4	4	2	2	2			
Estonia	1																		1
EEA[(a)]	11									3		1		5					
Finland	4												4						
France	27	1	2	1	7	3	1	2		1		3	4		1	2			1
Germany	49	14		7			4				6	2	2	5		6			1
Greece	221	16	11	11	5	11	10		13	8	28	17	38	12	17	11	3	2	8
Ireland	2															2			
Hungary	3	2			1														
Italy	278	27	39		7	3			13	17	57	23	28	7	29	18		2	8
Latvia	4	1		1			2												
Lithuania	17	7		3			3					4							
Moldova	9	9																	
Netherlands	135	17		1	2	10	2				5	10	33	13	26	10			6
Poland	42	17		4			3				3	10	2						3
Romania	11	2								1									8
Russia	9	8														1			
Slovenia	4																		4
Spain	6				1					3			1	1					
Sweden	28	5		4		5	5						7		2				
Ukraine	3	2									1								
UK	177	34		3	1	27	20					27	16	6	10	25			8
Elsewhere																			
Argentina	7				5												2		
Brazil	3																3		
Cambodia	2									2									
Canada	3											2							
China	21										6	1					14		
Egypt	2																2		
Georgia	1																	1	
India	14									13							1		
Japan	1										1								
Korea	1									1									
Malaysia	1										1								
Mexico	1																		1
Pakistan	7								2	5									
Surinam	3									3									
Thailand	8									8									
Turkey	25	1								1								23	
USA	10	1			2					6						1			
Viet Nam	2										1	1							
Unknown	**71**	**3**			**2**					**14**	**2**	**17**	**4**		**2**	**16**	**3**	**8**	

(a): EEA = European Economic Area.

6. Occurrence of sterigmatocystin in food

In total 1 259 samples were analysed by four laboratories in multiple analytical batches run between January and November 2014. Representative examples of extracted-ion chromatograms for samples in which sterigmatocystin was detected, both below and above 0.5 µg/kg, are provided in Appendix 3. In Table 9 the analysis results are summarized for the different categories of food products in this survey. The overall number of samples in which sterigmatocystin was detected was 124, 10 % of all samples analysed. Lower, middle and upper bound values for the average and median concentration of sterigmatocystin, and the 75th and 95th percentiles are included in Table 10. The highest values for the 95th percentiles (ignoring values resulting from sample numbers lower than 60) were obtained for the categories 'all cereal samples (excluding beer)' (0.88 µg/kg), processed rice (0.84 µg/kg), and breakfast cereals (0.83 µg/kg). Cereal grains seemed slightly more contaminated (more positives, higher levels) than cereal products. Within these groups, rice, oats and breakfast cereals accounted for the majority of the detections. More than 50 % of the samples positive for sterigmatocystin contained levels below the lowest validated level, i.e. in the range 0.050–0.15 to 0.5 µg/kg. 4.4 % of the samples contained sterigmatocystin at levels higher than 0.5 µg/kg. These levels were mainly between 0.5–5 µg/kg. Only two samples contained levels > 5 µg/kg: one rice (crop) sample (5.5 µg/kg) and one oats grain sample containing a relatively high level of 33 µg/kg.

The number of samples for each product is too low for an in-depth assessment of any relationship between country of origin, year of harvest, organic *vs* non-organic, or other variables and the levels of sterigmatocystin. This survey should be considered as a first snapshot on the occurrence of sterigmatocystin in food products grown and consumed in the EU. The brief discussion for each product type provided below should be read with this consideration in mind.

6.1. Cereal grains (except rice)

Sterigmatocystin was detected in all five types of cereals analysed, with incidental findings (2–6 %) in wheat, rye, maize, and barley, and a higher incidence in oats (22 %). Details on the contaminated samples are provided in Table 11. The higher incidence in oats was also observed by Uhlig et al. (2013). In their study, wheat, barley and oats from Norway, sampled in 2011 (unusual warm and wet) were analysed and sterigmatocystin was found in 7–15 % of the wheat and barley samples (up to 1.2 µg/kg), and 57 % of the oats samples (up to 20 µg/kg). In cereal samples taken in 2006–2007 in Latvia, on the other hand, Veršilovskis et al. (2008a), reported 25 % of the samples being contaminated, with the highest levels (> 25 µg/kg) in wheat and barley and a lower incidence in oats. For this reason, samples from Latvia/Lithuania (wheat, rye, oats) were included in the current survey. The results obtained show that in this survey, also for samples from that region, oats were the more contaminated type of cereal.

Table 9: Summary of analysis results for sterigmatocystin in food

Product	Samples analysed	Detected		LOD-0.5 µg/kg		0.5–1.5 µg/kg		1.5–5 µg/kg		> 5 µg/kg	
	N	N	%	N	%	N	%	N	%	N	%
OVERALL	**1 259**	**124**	**10 %**	**69**	**5 %**	**36**	**3 %**	**17**	**1.4 %**	**2**	**0.2 %**
ALL CEREALS (except beer)	**1 142**	**124**	**11 %**	**69**	**6 %**	**36**	**3 %**	**17**	**1.5 %**	**2**	**0.2 %**
CEREAL GRAINS	**429**	**55**	**13 %**	**22**	**5 %**	**19**	**4 %**	**12**	**3 %**	**2**	**0.5 %**
Wheat (soft)	169	10	6 %	8	5 %	2	1.2 %				
Wheat (hard/durum)	52	2	4 %	1	2 %	1	2 %				
Rye	35	2	6 %	1	3 %	1	3 %				
Maize	33	2	6 %			2	6 %				
Rice	28	27	96 %	8	29 %	8	29 %	10	36 %	1	4 %
Barley	59	1	2 %					1	2 %		
Oats	51	11	22 %	4	8 %	5	10 %	1	2 %	1	2 %
Spelt	2	0	0 %								
CEREAL PRODUCTS	**713**	**69**	**10 %**	**47**	**7 %**	**17**	**2.4 %**	**5**	**0.7 %**		
Products (to be processed)	**329**	**31**	**9 %**	**23**	**7 %**	**6**	**1.8 %**	**2**	**0.6 %**		
Grain milling products	125	6	5 %	5	4 %	1	0.8 %				
- wheat	85	*3*	4 %	2	2 %	1	1.2 %				
- rye	11	*0*	0 %								
- maize	18	*1*	6 %	1	6 %						
- rice	2	*0*	0 %								
- barley	1	*0*	0 %								
- oats	6	*2*	33 %	2	33 %						
- other	2	*0*	0 %								
Rice	89	19	21 %	12	13 %	5	6 %	2	2 %		
Pasta	115	6	5 %	6	5 %						
Products (consumed as such)	**384**	**38**	**10 %**	**24**	**6 %**	**11**	**3 %**	**3**	**0.8 %**		
Bread/rolls	143	10	7 %	6	4 %	2	1.4 %	2	1.4 %		
Breakfast cereals (incl. muesli)	97	18	19 %	11	11 %	6	6 %	1	1.0 %		
Fine bakery ware	90	6	7 %	5	6 %	1	1.1 %				
Cereal-based infant food	54	4	7 %	2	4 %	2	4 %				
BEER	**53**	**0**	**0 %**								
NUTS	**64**	**0**	**0 %**								
Peanuts	28	0	0 %								
Hazelnuts	36	0	0 %								

N = number, % = percentage.

Table 10: Average, median, 75th and 95th percentiles of the sterigmatocystin concentration (µg/kg) in different food categories

Product	Samples analysed	Detected	Average			Median			75th Percentile			95th Percentile[(a)]		
	N	%	LB	MB	UB	LB	MB	UB	LB	MB	UB	LB	MB	UB
OVERALL	**1 259**	**10 %**												
ALL CEREALS (except beer)	**1 142**	**11 %**	**0.10**	**0.15**	**0.21**	**0.00**	**0.050**	**0.10**	**0.00**	**0.050**	**0.10**	**0.10**	**0.25**	**0.50**
CEREAL GRAINS	**429**	**13 %**	**0.19**	**0.24**	**0.30**	**0.00**	**0.050**	**0.10**	**0.00**	**0.050**	**0.10**	**0.88**	**0.88**	**0.88**
Wheat (soft)	169	6 %	0.015	0.069	0.13	0.00	0.050	0.10	0.00	0.25	0.50	0.10	0.25	0.50
Wheat (hard/durum)	52	4 %	0.017	0.068	0.12	0.00	0.050	0.10	0.00	0.050	0.10	(0.035)	(0.12)	(0.24)
Rye	35	6 %	0.024	0.076	0.13	0.00	0.050	0.10	0.00	0.050	0.10	(0.23)	(0.35)	(0.55)
Maize	33	6 %	0.059	0.11	0.15	0.00	0.050	0.10	0.00	0.050	0.10	(0.84)	(0.84)	(0.84)
Rice (crop)	28	96 %	1.2	1.3	1.3	0.95	0.95	0.95	1.9	1.9	1.9	(4.2)	(4.2)	(4.2)
Barley	59	2 %	0.032	0.081	0.13	0.00	0.050	0.10	0.00	0.050	0.10	(0.00)	(0.050)	(0.10)
Oats	51	22 %	0.78	0.83	0.89	0.00	0.050	0.10	0.00	0.050	0.10	(1.7)	(1.7)	(1.7)
Spelt	2	0 %				0.00	0.050	0.10	0.00	0.050	0.10			
CEREAL PRODUCTS	**713**	**10 %**	**0.042**	**0.097**	**0.16**	**0.00**	**0.050**	**0.10**	**0.00**	**0.050**	**0.10**	**0.10**	**0.25**	**0.50**
Products (to be processed)	**329**	**9 %**	**0.032**	**0.087**	**0.15**	**0.00**	**0.050**	**0.10**	**0.00**	**0.050**	**0.10**	**0.10**	**0.25**	**0.50**
Grain milling products	125	5 %	0.008	0.060	0.11	0.00	0.050	0.10	0.00	0.050	0.10	0.00	0.050	0.10
Rice	89	21 %	0.10	0.15	0.23	0.00	0.050	0.10	0.00	0.050	0.10	0.84	0.84	0.84
Pasta	115	5 %	0.005	0.060	0.12	0.00	0.050	0.10	0.00	0.050	0.10	0.10	0.25	0.50
Products (consumed as such)	**384**	**10 %**	**0.051**	**0.11**	**0.17**	**0.00**	**0.050**	**0.10**	**0.00**	**0.050**	**0.10**	**0.10**	**0.25**	**0.50**
Bread/rolls	143	7 %	0.055	0.11	0.16	0.00	0.050	0.10	0.00	0.050	0.10	0.10	0.25	0.50
Breakfast cereals (incl. muesli)	97	19 %	0.093	0.15	0.22	0.00	0.050	0.10	0.00	0.050	0.10	0.83	0.83	0.83
Fine bakery ware	90	7 %	0.011	0.066	0.13	0.00	0.050	0.10	0.00	0.050	0.10	0.10	0.25	0.50
Cereal-based infant food	54	7 %	0.034	0.086	0.14	0.00	0.050	0.10	0.00	0.050	0.10	(0.26)	(0.38)	(0.56)
BEER	**53**	**0 %**	**0.00**	**0.005**	**0.010**	**0.00**	**0.005**	**0.010**	**0.00**	**0.005**	**0.010**	**(0.00)**	**(0.005)**	**(0.010)**
NUTS	**64**	**0 %**	**0.00**	**0.050**	**0.10**	**0.00**	**0.050**	**0.10**	**0.00**	**0.050**	**0.10**	**0.00**	**0.050**	**0.10**
Peanuts	28	0 %	0.00	0.050	0.10	0.00	0.050	0.10	0.00	0.050	0.10	(0.00)	(0.050)	(0.10)
Hazelnuts	36	0 %	0.00	0.050	0.10	0.00	0.050	0.10	0.00	0.050	0.10	(0.00)	(0.050)	(0.10)

N: number; %: percentage of the samples analysed in which sterigmatocystin was detected; LB: lower bound; MB: middle bound; UB: upper bound.
(a): when N < 60 then the calculated 95th percentile is in between brackets and should be considered as an indicative value only due to the limited number of data (EFSA, 2011).

Table 11: Cereal grain samples contaminated with sterigmatocystin

Product description (according to EFSA coding)	µg/kg[(a)]	Country of origin	Country of sampling	Year of production	Year of sampling
Wheat grain	1.3	UK	UK	2014	2014
Wheat grain, durum	(0.27)	Greece	Greece	2013	2014
Wheat grain, durum	0.79	Greece	Greece		2014
Wheat grain, soft	(0.15)	Greece	Greece	2013	2014
Wheat grain, soft	(0.30)	Greece	Greece	2013	2014
Wheat grain, soft	(0.23)	Italy	Italy	2013	2013
Wheat grain, soft	(0.25)	Italy	Italy	2013	2013
Wheat grain, soft	(0.27)	Italy	Italy	2013	2013
Wheat grain, soft	0.65	Italy	Italy	2014	2014
Wheat grain, soft	(0.06)	Lithuania	Lithuania	2013	2014
Wheat grain, soft	(0.32)	Poland	Poland	2014	2014
Wheat grain, soft	(0.29)	Sweden	Italy	2013	2013
Barley grain	1.9	Greece	Greece		2014
Maize grain	0.64	France	Italy	2013	2013
Maize grain	1.3	France	Greece	2013	2014
Rye grain	0.75	Greece	Greece		2014
Rye grain	(0.27)	Sweden	Italy	2013	2013
Oats grain	2.8	Greece	Greece	2014	2014
Oats grain	0.57	Latvia	Latvia	2013	2014
Oats grain	0.60	Latvia	Latvia	2013	2014
Oats grain	(0.38)	Lithuania	Latvia	2013	2014
Oats grain	33	Netherlands	Netherlands	2013	2014
Oats grain	(0.17)	Poland	Poland	2014	2014
Oats grain	(0.20)	Poland	Poland	2014	2014
Oats grain	(0.28)	Sweden	Italy	2013	2013
Oats grain	0.63	UK	UK	2013	2014
Oats grain[(b)]	0.67	UK	UK	2013	2014
Oats grain	0.97	UK	UK	2013	2014

(a): between brackets: below lowest validated concentration, values indicative.
(b): organic.

6.2. Rice

Rice was clearly identified in this survey as the product with the highest incidence of sterigmatocystin contamination. Details on the contaminated samples are provided in Table 12. All samples of non-processed rice taken in Italy in 2013 (mostly from the same region) were contaminated (0.2–1.9 µg/kg). The same was observed for rice grown in Greece and sampled in 2014 (0.5–5.5 µg/kg). Processed ('consumer ready') rice was less contaminated. Rice from Europe seemed more contaminated than rice from Asia/North-South America (Figure 7). In the literature potential contamination of rice with sterigmatocystin has been reported. High levels have been found in the past, but in a survey done in Japan none of the 48 samples analysed were contaminated (Tanaka et al., 2007). No recent surveys of rice for sterigmatocystin could be found in the literature to compare with the results from this survey.

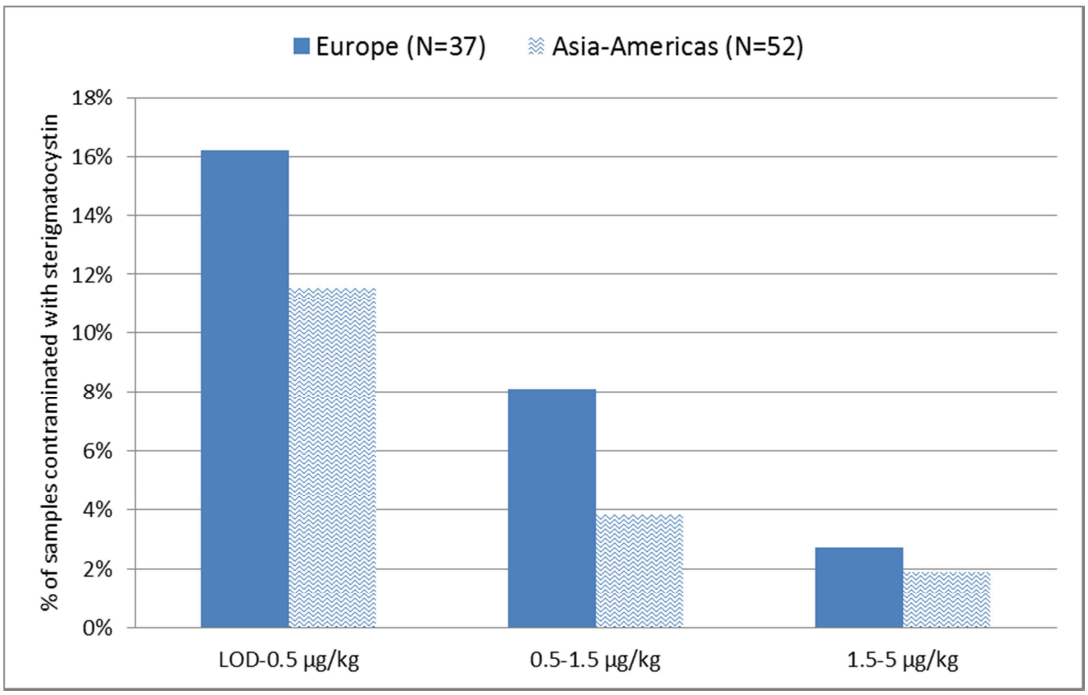


Figure 7: Contamination of processed rice with sterigmatocystin from in- and outside Europe

Table 12: Cereal grain samples contaminated with sterigmatocystin

Product description (according to EFSA coding)	µg/kg[(a)]	Country of origin	Country of sampling	Year of production	Year of sampling
Rice (Crop)	(0.49)	Greece	Greece	2014	2014
Rice (Crop)	0.56	Greece	Greece	2014	2014
Rice (Crop)	0.69	Greece	Greece	2014	2014
Rice (Crop)	1.4	Greece	Greece	2014	2014
Rice (Crop)	1.5	Greece	Greece	2014	2014
Rice (Crop)	1.8	Greece	Greece	2014	2014
Rice (Crop)	1.8	Greece	Cyprus	2014	2014
Rice (Crop)	1.9	Greece	Greece	2014	2014
Rice (Crop)	2.3	Greece	Greece	2014	2014
Rice (Crop)	2.6	Greece	Greece	2014	2014
Rice (Crop)[(b)]	2.7	Greece	Greece	2014	2014
Rice (Crop)	5.5	Greece	Greece	2014	2014
Rice (Crop)[(b)]	(0.21)	Italy	Italy	2013	2014
Rice (Crop)[(b)]	(0.21)	Italy	Italy	2013	2014
Rice (Crop)	(0.22)	Italy	Italy	2013	2014
Rice (Crop)	(0.32)	Italy	Italy	2013	2014
Rice (Crop)	(0.41)	Italy	Italy	2013	2014
Rice (Crop)	0.65	Italy	Italy	2013	2014
Rice (Crop)	0.86	Italy	Italy	2013	2014
Rice (Crop)	0.90	Italy	Italy	2013	2014
Rice (Crop)[(b)]	1.0	Italy	Italy	2013	2014
Rice (Crop)	1.2	Italy	Italy	2013	2014
Rice (Crop)	1.8	Italy	Italy	2013	2014
Rice (Crop)	1.9	Italy	Italy	2013	2014
Rice (Crop)	1.9	Italy	Italy	2013	2014
Rice (Crop)	(0.14)	Pakistan	Greece	2014	2014
Rice (Crop)	(0.21)	Pakistan	Greece	2014	2014
Rice[(b)]	(0.11)	EEA	Netherlands		2014
Rice, brown	(0.15)	Italy	Netherlands		2013
Rice, brown	(0.23)	United States	Netherlands		2014
Rice, brown	(0.35)	United States	Netherlands		2014
Rice, brown[(b)]	0.56	Unknown	UK		2014
Rice, brown	1.0	Unknown	UK		2014
Rice, long-grain	(0.06)	Suriname	Netherlands		2014
Rice, long-grain	(0.12)	Suriname	Netherlands		2013
Rice, long-grain[(b)]	(0.29)	India	Greece		2014
Rice, long-grain	(0.39)	India	Greece		2014
Rice, long-grain	0.64	EU	UK		2014
Rice, parboiled	1.1	Greece	Greece	2014	2014
Rice, parboiled	1.7	Unknown	Germany		2014
Rice, parboiled	2.2	Spain	Netherlands		2013
Rice, white	(0.13)	Greece	Greece		2014
Rice, white	(0.14)	Greece	Greece		2014
Rice, white	(0.20)	Italy	Italy	2013	2014
Rice, white	(0.24)	Cyprus	Cyprus		2013
Rice, white	0.68	Cyprus	Cyprus		2013

EEA: European Economic Area; EU: European Union.
(a): between brackets: below lowest validated concentration, values indicative.
(b): organic.

6.3. Cereal products except rice, not consumed as such

In the category cereal products that are not consumed as such but only after cooking or baking, 5 % of the samples were found to be contaminated with sterigmatocystin (for details see Table 13). With one exception, levels were all below 0.5 µg/kg. The detection of sterigmatocystin in oats and spelt based products are noteworthy, given the low number of these samples included in this category.

6.4. Cereal products, consumed as such

This category included a range of products that are consumed as such or after mixing with other foodstuffs (e.g. milk). Some products contained single ingredients whereas others were composed of many ingredients (see Table 14). A similar detection rate (7 %) was observed for bread, fine bakery ware and cereal-based infant food (7 %), and a higher incidence in breakfast cereals and muesli (19 %). Especially for the latter category, rice and oats were over-represented in the ingredients list of the contaminated samples, which corresponds to the higher incidence of sterigmatocystin in rice and oats grain. The contaminated cereal-based infant foods all contained rice as a (major) ingredient, again in line with the findings in rice. For bread and fine bakery ware, the link with contaminated ingredients was less obvious.

6.5. Beer

The beer samples analysed in this survey were mostly regular beers, since this best reflected consumption of the average consumer. Due to the different sample preparation, the LOD for beer was lower (0.005–0.01 µg/kg) than for the other matrices. In the recent literature, the detection of sterigmatocystin in beer has been reported by Veršilovskis et al. (2008b). In that study two out of 26 Latvian beers were contaminated (4–8 µg/kg, one light, one dark beer). In the current survey, sterigmatocystin was not identified in any of the samples.

6.6. Nuts

Two types of nuts were included in the current survey. Peanuts originated from outside Europe, mainly from China. Hazelnuts were almost exclusively originating from Turkey. In none of the samples sterigmatocystin was detected. This was in contrast to a recent study by Varga et al. (2013) who found sterigmatocystin in five out of 15 samples of peanuts (all below the LOQ of 2.4 µg/kg, origin Turkey, 2007), and in 21 out of 22 hazelnut samples (up to 5.5 µg/kg, origin Turkey, 2007). In hazelnuts samples collected in Austria, the same research group did not detect sterigmatocystin, with the exception of one sample collected in 2009 (0.92 µg/kg) (Varga E, University of Natural Resources and Life Sciences, Vienna, personal communication November 2014).

Table 13: Cereal products (not consumed as such, to be cooked before consumption) contaminated with sterigmatocystin

Product description (according to EFSA coding)	µg/kg[(a)]	Country of origin	Country of sampling	Ingredients[(b)]
Wheat flour, white	(0.21)	Greece	Greece	
wheat flour white	(0.25)	Italy	Italy	[2013]
Wheat flour, white	0.66	Italy	Italy	[2013]
Corn flour	(0.05)	Netherlands	Netherlands	
Oat flour	(0.11)	Unknown	Netherlands	
Oat groats	(0.06)	Netherlands	Netherlands	
Noodle, wheat flour, without eggs[(c)]	(0.24)	Japan	Netherlands	wholemeal wheat flour
Pasta, wheat flour, with eggs	(0.12)	Netherlands	Netherlands	wheat flour, eggs, tapioca flour
Pasta, wheat flour, without eggs	(0.17)	Greece	Greece	durum wheat [2013]
Pasta, wheat flour, without eggs	(0.21)	Italy	Italy	durum wheat [2012]
Pasta wheat flour without eggs	(0.30)	Italy	Italy	durum wheat [2013]
Pasta, spelt flour	(0.22)	Germany	Netherlands	wholemeal spelt, eggs (10 %)

(a): between brackets: below lowest validated concentration, indicative values.
(b): between brackets: year of production if known.
(c): organic.

Table 14: Cereal products (not consumed as such, to be cooked before consumption) contaminated with sterigmatocystin

Product description (according to EFSA coding)	µg/kg[(a)]	Country of origin	Country of sampling	Ingredients[(b)]
Wheat bread, white	0.84	Greece	Greece	wheat flour, sesame seeds
Wheat bread, brown	(0.17)	Netherlands	Netherlands	
Wheat toast bread, white	1.7	Greece	Greece	wheat flour (55 %), natural sourdough, butter (5.9 %), butter, sugar
Multigrain bread	(0.34)	Netherlands	Netherlands	wheat, oats, barley, rye, malt
Multigrain bread	(0.39)	Italy	Italy	wheat flour (durum, type00 65,7 %), wheat flour (soft type00), cereals flour [2013]
Multigrain rolls	(0.23)	Italy	Italy	brown rice (69,8 %), oats (30 %) [2013]
Crisp bread, rye wholemeal	3.7	Unknown	UK	wholemeal rye flour
Crisp bread, wheat, light	(0.18)	Poland	Poland	wheat flour, sugar, vegetable fat, soya, whey powder
Rice bread[(c)]	1.1	Italy	Italy	brown rice 99.7 % [2013]
Croutons	(0.32)	Sweden	Italy	wheat flour (84 %), sesame seeds (10 %), vegetable fat [2012]
Cereal flakes	(0.20)	Italy	Italy	wheat bran 87 % [2014]
Corn flakes	(0.20)	Greece	Greece	corn
Corn flakes	(0.26)	Greece	Greece	corn
Corn flakes with sugar	(0.24)	Cyprus	Cyprus	corn flour, cocoa
Mixed cereal flakes	(0.12)	unknown	Netherlands	rice (45 %), wholemeal wheat (32 %), barley (8 %), malted barley flour, sugar
Mixed cereal flakes	(0.12)	Germany	Netherlands	rice (63.8 %), sugar, wholemeal wheat (7.4 %), wheat gluten, rice starch, molasses, skimmed milk powder, wheat germs, barley malt extract
Mixed cereal flakes	(0.34)	Italy	Italy	oat flour (22 %), wholemeal rice (19 %), wholemeal wheat (13 %) [2014]
Mixed cereal flakes	0.81	Italy	Italy	wheat bran (75 %), corn [2014]
Oat flakes	0.60	UK	UK	[2014]
Oat flakes, wholemeal[(c)]	1.0	European Union	Germany	
Rice flakes[(c)]	(0.13)	Italy	Netherlands	wholemeal rice
Rice flakes	(0.34)	France	Italy	rice (60 %), wholemeal wheat (15 %) [2014]
Wheat flakes	0.68	Italy	Italy	wholemeal wheat (67 %), wheat bran (21 %) [2014]

(a): between brackets: below lowest validated concentration, values indicative.
(b): between brackets: year of production if known.
(c): organic.

Table 14 (continued): Cereal products (not consumed as such, to be cooked before consumption) contaminated with sterigmatocystin

Product description (according to EFSA coding)	µg/kg[(a)]	Country of origin	Country of sampling	Ingredients[(b)]
Muesli with chocolate	2.4	Unknown	Netherlands	rolled oats (38 %), chocolate (13 %), sunflower oil (11 %), wheat (9.8 %), rice flour (2.1 %), coconut
Cereal bar with added sugar	0.25	Italy	Italy	wholemeal oats (49 %), rice flour [2013]
Popped cereals	1.0	Italy	Italy	cereals (56 %, oat meal, wheat flour, ground rice, semolina corn), honey (5 %) [2013]
Wheat, popped, with sugar	0.25	Greece	Greece	wheat flour, cocoa powder, sugar, syrup, barley
Oat porridge	1.4	UK	UK	rolled oats.
Croissant, filled with chocolate	0.23	Cyprus	Cyprus	wheat flour, cocoa, vegetable oil
Croissant, filled with chocolate	0.23	Greece	Greece	wheat flour, cocoa, vegetable oil
Croissant, filled with chocolate	0.50	Greece	Greece	wheat flour, cocoa cream (33 %), milk powder (7 %), soya lecithin, margarine, eggs, sugar, pistachio, sesame
Biscuits, sweet, plain	0.27	Italy	Italy	oat flakes (34 %), wholemeal (32 %), fruit (9.7 %) [2013]
Biscuits, sweet, plain[(c)]	0.27	Italy	Italy	wheat flour, wheat bran, eggs [2013]
Biscuits, sweet, plain	0.36	Netherlands	Netherlands	wheat flour (13 %), sunflower seeds, sugar, sesame seed (6 %), linseed (6 %), gluten powder, glucose syrup, barley malt powder, yeast, egg, fructose, milk powder, rapeseed oil, wheat gluten, coconut oil
cereal-based infant food[(d)]	0.20	Netherlands	Netherlands	rice flour
cereal-based infant food[(c ,d)]	0.39	Germany	Netherlands	wholemeal rice flour (70 %), maize flour (20 %), wholemeal millet flour (10 %)
cereal-based infant food[(d)]	0.75	European Union	UK	cereal flour (wheat, oats, rice, millet, barley, maize, rye), banana
cereal-based infant food[(c, d)]	0.90	Netherlands	Netherlands	brown rice flour, buckwheat flour

(a): between brackets: below lowest validated concentration, indicative values.
(b): between brackets: year of production if known.
(c): organic.
(d): Cereal-based infant food all belonged to the EFSA product code: 'Simple cereals which are or have to be constituted with milk or other appropriate nutritious liquids'.

CONCLUSIONS AND RECOMMENDATIONS

CONCLUSIONS

- LC-MS/MS-based methods for the determination of sterigmatocystin in various food matrices were successfully validated at levels down to 0.5 µg/kg in four laboratories, which was below the 1.5 µg/kg required by EFSA to facilitate risk assessment.
- The methods enabled highly sensitive detection of sterigmatocystin in food with LODs in the range of 0.05–0.15 µg/kg for grain, cereal products and nuts, and 0.005–0.01 µg/kg for beer.
- The methods proved to be robust during analysis of the survey samples and acceptable average recoveries (81–107 %) and reproducibilities (RSD_R, better than 20 %) were obtained.
- Sterigmatocystin exceeded 1.5 µg/kg in 1.4 % of all food products analysed in this survey, and was detectable in 10 % of the 1259 samples analysed.
- Rice and oats grains were most prone to contamination with sterigmatocystin.
- In processed cereal products the occurrence of sterigmatocystin was slightly lower, 9–10 % compared to a 13 % detection rate in cereal grains.
- In line with the more frequent contamination of rice and oats, products containing these ingredients are also more often contaminated with sterigmatocystin.
- No sterigmatocystin was detected in beer, peanuts and hazelnuts.
- Rice and breakfast cereals are relevant products with respect to potential exposure of the consumer to sterigmatocystin.

RECOMMENDATIONS

- Mycotoxin formation depends on various factors including climatic conditions. This study reflects the situation of (little over) one year of sampling (2013–2014). Therefore a follow up study would be necessary to obtain information on seasonal variations.
- Since rice flour is an important ingredient in cereal-based infant food, and oats to a lesser extent, a more extensive survey of these products when containing rice and/or oats as major ingredient is considered relevant.
- More data are required on the occurrence of sterigmatocystin in rice grown in the EU, and on the effect of processing of the rice after harvest on the levels.

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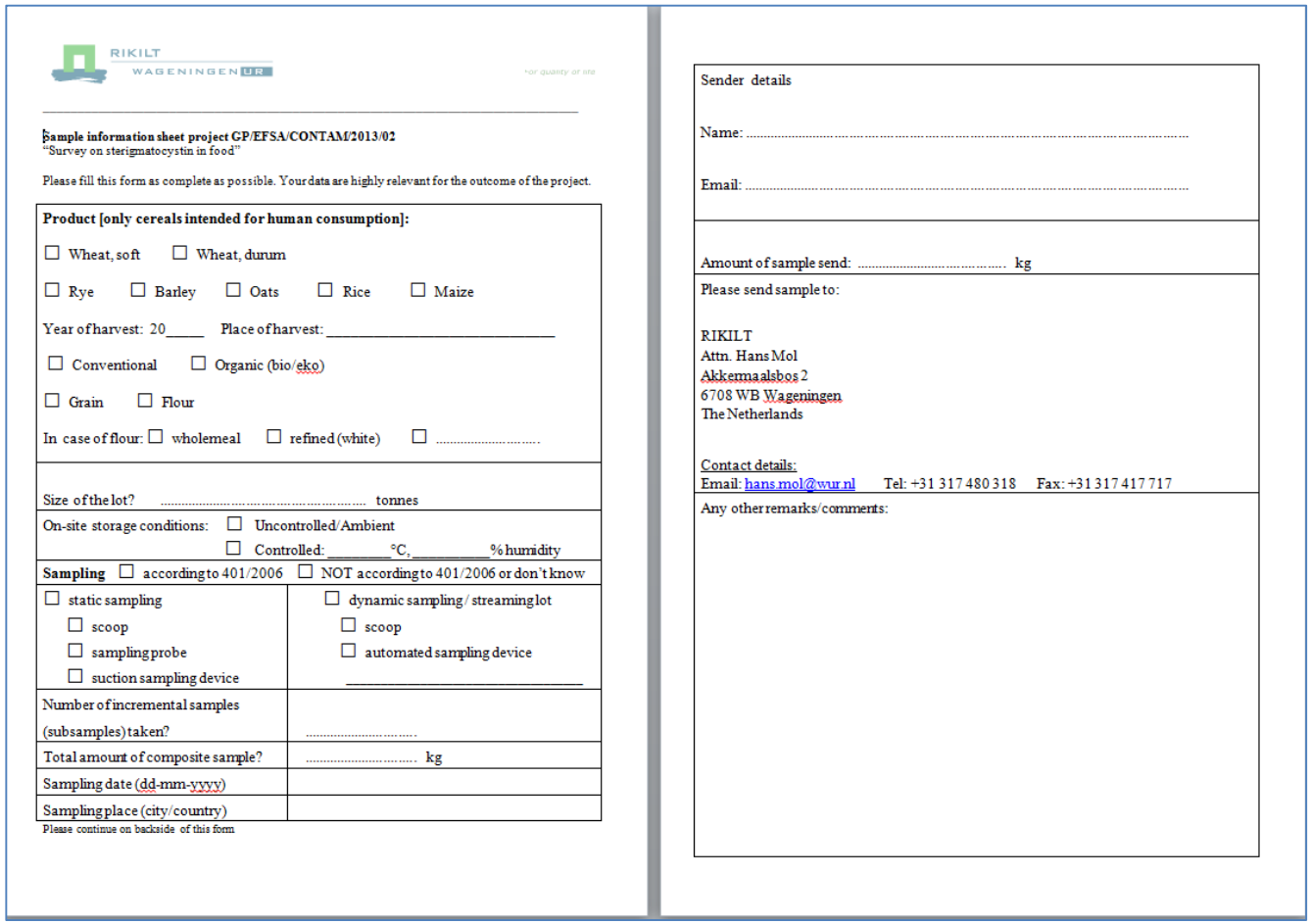
APPENDICES

Appendix 1. Example sample information sheet used for external partners



RIKILT WAGENINGEN UR

Sample information sheet project GP/EFSA/CONTAM/2013/02
"Survey on sterigmatocystin in food"

Please fill this form as complete as possible. Your data are highly relevant for the outcome of the project.

Product [only cereals intended for human consumption]:

☐ Wheat, soft ☐ Wheat, durum

☐ Rye ☐ Barley ☐ Oats ☐ Rice ☐ Maize

Year of harvest: 20____ Place of harvest: _____

☐ Conventional ☐ Organic (bio/eko)

☐ Grain ☐ Flour

In case of flour: ☐ wholemeal ☐ refined (white) ☐ .....

Size of the lot? ..... tonnes

On-site storage conditions: ☐ Uncontrolled/Ambient
☐ Controlled: _____°C; _____% humidity

Sampling ☐ according to 401/2006 ☐ NOT according to 401/2006 or don't know

☐ static sampling ☐ scoop ☐ sampling probe ☐ suction sampling device	☐ dynamic sampling / streaming lot ☐ scoop ☐ automated sampling device _____
Number of incremental samples (subsamples) taken?	.....
Total amount of composite sample?	..... kg
Sampling date (dd-mm-yyyy)	
Sampling place (city/country)	

Please continue on backside of this form

Sender details

Name: .....

Email: .....

Amount of sample send: ..... kg

Please send sample to:

RIKILT
Attn. Hans Mol
Akkermaalsbos 2
6708 WB Wageningen
The Netherlands

Contact details:
Email: hans.mol@wur.nl Tel: +31 317 480 318 Fax: +31 317 417 717

Any other remarks/comments:

Appendix 2. Analytical Quality Control data

Date	RIKILT sample code	Matrix	Recovery[(a)]
07/02/2014	200323906	QC incurred rice (feed)	2.00[(b)]
07/02/2014	79497478	Infant food (cereal based)	103 %
07/02/2014	76490635	Wheat (slurry)	104 %
07/02/2014	76203504	Rice (slurry)	105 %
07/02/2014	79218413	Maize	104 %
07/02/2014	wheat slurry (fresh spike)	Wheat (slurry)	100 %
07/02/2014	wheat slurry (storage stability spike Jan2014)	Wheat (slurry)	95 %
08/05/2014	200331723	Biscuit (slurry)	109 %
08/05/2014	200331735	Bread (slurry)	93 %
08/05/2014	200331747	Bread(slurry)	94 %
08/05/2014	wheat slurry (fresh spike)	Wheat (slurry)	99 %
08/05/2014	wheat slurry (storage stability spike Jan2014)	Wheat (slurry)	94 %
02/06/2014	200335769	Pasta spaghetti (slurry)	105 %
02/06/2014	200335780	Pasta gnocchi (slurry)	110 %
02/06/2014	200335785	Pasta (mie) (slurry)	108 %
02/06/2014	200337849	Muesli (slurry)	98 %
02/06/2014	200337856	Cornflakes (slurry)	103 %
02/06/2014	200337861	Muesli (slurry)	113 %
27/06/2014	200337336	Wheat flour	100 %
27/06/2014	200337345	Wheat grain (slurry)	97 %
27/06/2014	200337356	Oats grain (slurry)	101 %
27/06/2014	wheat slurry (fresh spike)	Wheat (slurry)	92 %
30/06/2014	200340179	Wheat (slurry)	101 %
30/06/2014	200340195	Rice (slurry)	98 %
30/06/2014	200340234	Rice (slurry)	111 %
30/06/2014	wheat slurry (fresh spike)	Wheat (slurry)	103 %
30/06/2014	wheat slurry (storage stability spike Jan2014)	Wheat (slurry)	93 %
14/08/2014	200343532	Biscuit (slurry)	103 %
14/08/2014	200343543	Wheat (slurry)	107 %
14/08/2014	200343553	Biscuit (slurry)	103 %
14/08/2014	200344996	Oat flour	116 %
14/08/2014	200341554	Pasta (macaroni)	114 %
16/09/2014	200341554	Pasta (macaroni)	115 %
16/09/2014	200345331	Rice	85 %
16/09/2014	200348155	Oats flakes (slurry)	119 %
16/09/2014	200348270	Wheat (slurry)	114 %
16/09/2014	wheat slurry (fresh spike)	Wheat (slurry)	114 %
16/09/2014	wheat slurry (storage stability spike Jan2014)	Wheat (slurry)	114 %
16/09/2014	200348989	Infant food (rice flour)	99 %
16/09/2014	200349380	Wheat (slurry)	110 %
16/09/2014	200349654	Oats bran (slurry)	126 %
16/09/2014	wheat slurry (fresh spike)	Wheat (slurry)	112 %
31/10/2014	200353109	Oats (slurry)	109 %
31/10/2014	wheat slurry (fresh spike)	Wheat (slurry)	104 %
31/10/2014	wheat slurry (storage stability spike Jan2014)	Wheat (slurry)	104 %
Cereals grains		**Average (RSD_R%, N)**	**103 % (7 %, N=25)**
Cereal products		**Average (RSD_R%, N)**	**107 % (8 %, N=19)**
Cereals all		**Average (RSD_R%, N)**	**105 % (8 %, N=44)**

N: number; RSD_R: Relative standard deviation under reproducibility conditions.
(a): samples spiked at 1.5 µg/kg.
(b): naturally contaminated rice (feed) sample.

Date	UCSC sample code	Matrix	Recovery[(a)]
25/02/2014	sterigbirra-11	Beer	91 %
17/10/2014	cerealsterig-469	Beer	92 %
04/06/2014	cerealsterig-297	Barley	94 %
04/06/2014	cerealsterig-299	Maize	90 %
24/06/2014	cerealsterig-409	Maize	90 %
30/04/2014	cerealsterig-186	Oat	92 %
07/05/2014	cerealsterig-239	Oat	92 %
30/04/2014	cerealsterig-185	Rye	93 %
07/05/2014	cerealsterig-237	Rye	94 %
04/06/2014	cerealsterig-296	Rye	92 %
19/02/2014	frumsterigm-39	Wheat	95 %
30/04/2014	cerealsterig-184	Wheat	94 %
07/05/2014	cerealsterig-238	Wheat	93 %
04/06/2014	cerealsterig-295	Wheat	93 %
17/10/2014	cerealsterig-468	Wheat	95 %
04/06/2014	cerealsterig-300	Pasta	94 %
10/06/2014	cerealsterig-348	Pasta	94 %
04/06/2014	cerealsterig-298	Bread	94 %
10/06/2014	cerealsterig-347	Bread	93 %
10/06/2014	cerealsterig-349	Breakfast cereals	91 %
24/06/2014	cerealsterig-407	Infant food (cereal based)	93 %
12/03/2014	cerealsterig-31	Wheat flour	94 %
24/06/2014	cerealsterig-408	Wheat flour	94 %
Cereal grains		**Average (RSD_R%, N)**	**93 % (2 %, N=13)**
Cereal products		**Average (RSD_R%, N)**	**93 % (1 %, N=8)**
Cereals all		**Average (RSD_R%, N)**	**93 % (2 %, N=21)**
Beer		**Average**	**92 % (N=2)**

N: number; RSD_R: Relative standard deviation under reproducibility conditions; UCSC: Università Cattolica del Sacro Cuore., Italy.
(a): cereal samples spiked at 1.5 µg/kg, beer at 0.5 µg/kg

Date	Fera sample code	Matrix	Recovery[(a)]
11/07/2014	QC Spike @ 1.5µg/kg S13-063058	Wheat	96 %
11/07/2014	QC Spike @ 1.5µg/kg S14-041310	Pasta	104 %
11/07/2014	QC Spike @ 1.5µg/kg S14-011052	Infant food	95 %
11/07/2014	QC Spike @ 1.5µg/kg S14-010942	Rice	101 %
20/08/2014	QC Spike @ 1.5µg/kg S13-063058	Wheat	78 %
20/08/2014	QC Spike @ 1.5µg/kg S14-041310	Pasta	93 %
20/08/2014	QC Spike @ 1.5µg/kg S14-43557	Barley	86 %
20/08/2014	QC Spike @ 1.5µg/kg S14-03567	Barley	120 %
02/10/2014	QC Spike @ 1.5µg/kg S13-063058	Wheat	82 %
02/10/2014	QC Spike @ 1.5µg/kg S14-041310	Pasta	107 %
02/10/2014	QC Spike @ 1.5µg/kg S14-048738	Pearl barley	110 %
02/10/2014	QC Spike @ 1.5µg/kg S14-048749	Pasta	83 %
08/10/2014	QC Spike @ 1.5µg/kg S13-063058	Wheat	108 %
08/10/2014	QC Spike @ 1.5µg/kg S14-041310	Pasta	104 %
08/10/2014	QC Spike @ 1.5µg/kg S14-042605	Oat	88 %
08/10/2014	QC Spike @ 1.5µg/kg S14-048779	Rice	120 %
16/10/2014	QC Spike @ 1.5µg/kg S13-063058	Wheat	114 %
16/10/2014	QC Spike @ 1.5µg/kg S14-050033	Noodles	115 %
16/10/2014	QC Spike @ 1.5µg/kg S14-050057	Cracker	87 %
16/10/2014	QC Spike @ 1.5µg/kg S14-050053	Pasta	136 %
16/10/2014	QC Spike @ 1.5µg/kg S14-048788	Rice	102 %
16/10/2014	QC Spike @ 1.5µg/kg S14-050056	Rye	107 %
16/10/2014	QC Spike @ 1.5µg/kg S14-011024	Infant food	116 %
24/10/2014	QC Spike @ 1.5µg/kg S13-063058	Wheat	113 %
24/10/2014	QC Spike @ 1.5µg/kg S14-041310	Pasta	89 %
24/10/2014	QC Spike @ 1.5µg/kg S14-050851	Wheat	116 %
24/10/2014	QC spike @ 3.0µg/kg S14-042615	Muesli (slurry)	114 %
31/10/2014	QC Spike @ 1.5µg/kg S13-063058	Wheat	85 %
31/10/2014	QC Spike @ 1.5µg/kg S14-041310	Pasta	78 %
31/10/2014	QC Spike @ 1.5µg/kg S14-051240	Maize	85 %
14/11/2014	QC Spike @ 1.5µg/kg S13-063058	Wheat	85 %
14/11/2014	QC Spike @ 1.5µg/kg S14-041310	Pasta	84 %
14/11/2014	QC Spike @ 1.5µg/kg AS14-051277-003	Oat	83 %
14/11/2014	QC Spike @ 1.5µg/kg AS14-051281-003	Rye	88 %
24/11/2014	QC Spike @ 1.5µg/kg S13-063058	Wheat	103 %
24/11/2014	QC Spike @ 1.5µg/kg S14-041310	Pasta	106 %
24/11/2014	QC Spike @ 1.5µg/kg S14-052948	Biscuits	98 %
24/11/2014	QC Spike @ 1.5µg/kg S14-052938	Crispbread	107 %
24/11/2014	QC Spike @ 1.5µg/kg S14-052932	Bread	99 %
Cereal grains		**Average (RSD_R%, N)**	**99 % (14 %, N=21)**
Cereal products		**Average (RSD_R%, N)**	**101 % (14 %, N=18)**
Cereals all		**Average (RSD_R%, N)**	**100 % (14 %, N=39)**

N: number; Fera: Food and Environment Research Agency, UK; RSD_R: Relative standard deviation under reproducibility conditions.
(a): cereal samples spiked at 1.5 µg/kg.

Date	Fera sample code	Matrix	Result (µg/kg)[(a)]
11/07/2014	S14-041306_QC	QC incurred rice (feed)	1.90
20/08/2014	S14-041306_QC	QC incurred rice (feed)	2.21
02/10/2014	S14-041306_QC	QC incurred rice (feed)	2.11
08/10/2014	S14-041306_QC	QC incurred rice (feed)	3.08
16/10/2014	S14-041306_QC	QC incurred rice (feed)	1.99
24/10/2014	S14-041306_QC	QC incurred rice (feed)	2.00
31/10/2014	S14-041306_QC	QC incurred rice (feed)	1.77
14/11/2014	S14-041306_QC	QC incurred rice (feed)	1.57
24/11/2014	S14-041306_QC	QC incurred rice (feed)	1.98
QC incurred rice (feed)		**Average (RSD_R%, N)**	**2.07 (20 %, N=9)**

N: number; Fera: Food and Environment Research Agency, UK; RSD_R: Relative standard deviation under reproducibility conditions.
(a): same sample as in Table 5.

Date	BPI sample code	Matrix	Recovery[(a)]
27/05/2014	17	Hazelnut	74 %
27/05/2014	18	Peanut	81 %
18/07/2014	36	Hazelnut	92 %
18/07/2014	37	Peanut	87 %
25/10/2014	48	Hazelnut	72 %
Nuts		**Average (RSD_R%, N)**	**81 % (10 %, N=5)**

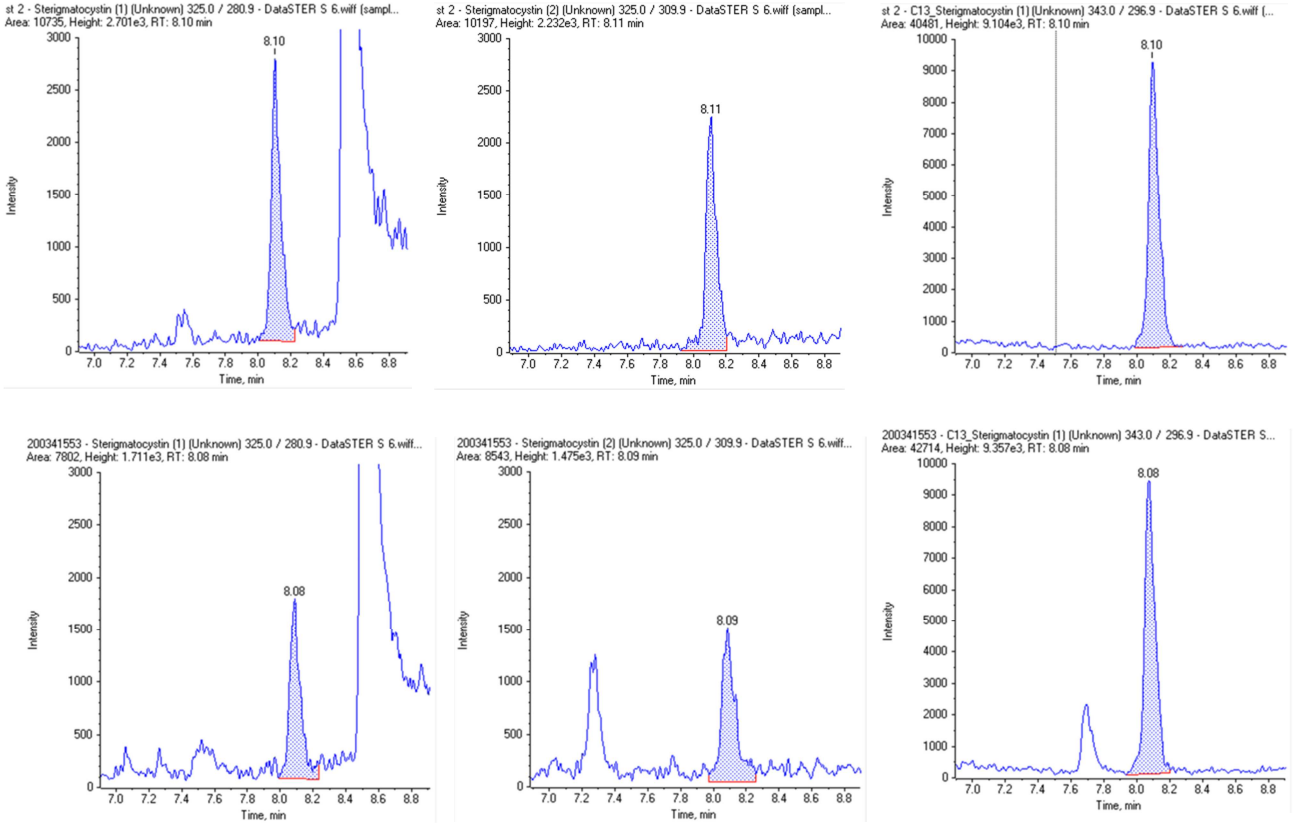
BPI: Benaki Phytopathological Institute, Greece; N: number; RSD_R: Relative standard deviation under reproducibility conditions.
(a): nut samples spiked at 1.5 µg/kg.

Date	BPI sample code	Matrix	Recovery[(a)]
13/01/2014	1	Bread	98 %
13/01/2014	2	Rice	104 %
22/01/2014	4	Bread	77 %
22/01/2014	5	Bread	78 %
22/01/2014	6	Bread	113 %
22/01/2014	7	Bread	72 %
22/01/2014	8	Bread	84 %
22/01/2014	3	Rice	106 %
11/03/2014	9	Bread	98 %
11/03/2014	12	Croissant	81 %
11/03/2014	14	Oat	83 %
11/03/2014	10	Pasta	93 %
11/03/2014	16	Pasta	87 %
11/03/2014	15	Wheat	113 %
27/05/2014	24	baby	122 %
27/05/2014	26	Corn flakes	87 %
27/05/2014	21	Fine bakery ware	123 %
27/05/2014	25	Flour	74 %
27/05/2014	19	Noodles	88 %
27/05/2014	22	Pasta	79 %
27/05/2014	23	Rye	86 %
27/05/2014	27	Trachanas	91 %
27/05/2014	20	Wheat	72 %
30/05/2014	31	Barley	83 %
30/05/2014	28	Bread (toast)	74 %
30/05/2014	29	Croissant	70 %
30/05/2014	30	Oat	74 %
07/07/2014	32	Pasta	71 %
07/07/2014	33	Wheat	86 %
18/07/2014	35	Flour	92 %
18/07/2014	38	Pasta	70 %
18/07/2014	39	Pasta	71 %
18/07/2014	34	Toast bread	87 %
24/10/2014	45	Barley	80 %
24/10/2014	41	Oat	95 %
24/10/2014	42	Rice	97 %
24/10/2014	44	Rye	92 %
24/10/2014	43	Wheat durum	84 %
24/10/2014	40	Wheat soft	72 %
25/10/2014	46	Corn	72 %
25/10/2014	47	Rice	71 %
Cereal grains		**Average (RSD_R%, N)**	**86 % (15 %, N=17)**
Cereal products		**Average (RSD_R%, N)**	**87 % (18 %, N=24)**
Cereals all		**Average (RSD_R%, N)**	**87 % (16 %, N=43)**

BPI: Benaki Phytopathological Institute, Greece; N: number; RSD_R: Relative standard deviation under reproducibility conditions.
(a): cereal samples spiked at 1.5 µg/kg.

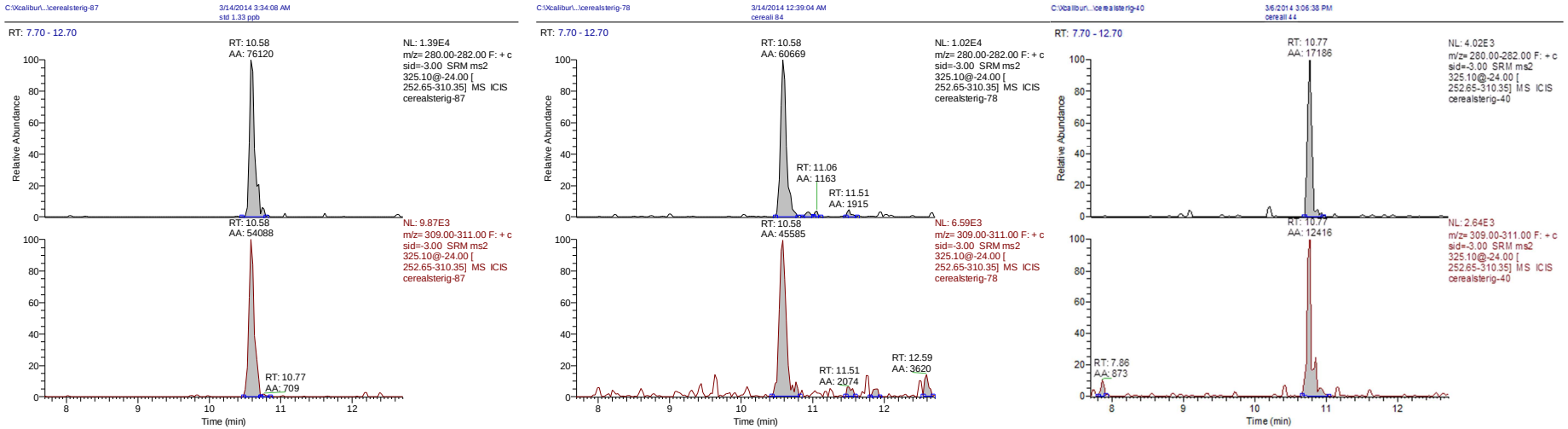
Appendix 3. Example chromatograms of positive samples from the survey

RIKILT



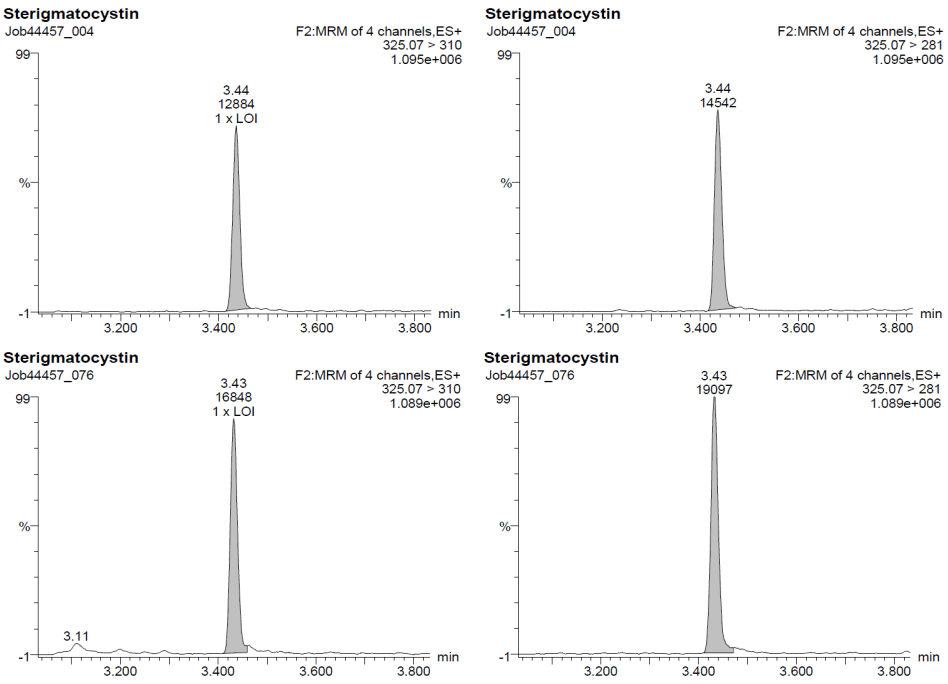
Top row: solvent standard (0.031 ng/mL) transition m/z 325→281, 325→310; 343→297 (^{13}C-label). Corresponding to 0.25 µg/kg (^{13}C-label 1.5 µg/kg).
Bottom row: sample Crisp bread, wheat, light containing sterigmatocystin at 0.18 µg/kg.

Università Cattolica del Sacro Cuore – UCSC, Italy



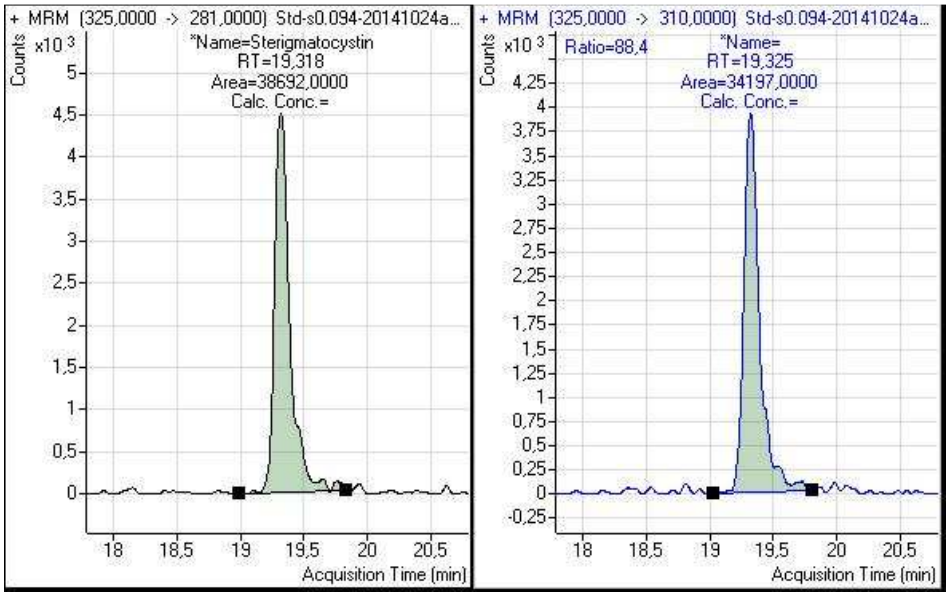
Left. Calibration standard (1.33 ng/mL of sterigmatocystin), corresponding to 2.1 ng/g in cereal samples; m/z 325→281 (upper) and m/z 325→310 (lower).
Middle. Paddy rice sample in which sterigmatocystin was quantified (calculated level = 1.9 ng/g); m/z 325→281 (upper) and m/z 325→310 (lower).
Right. Bread/roll sample in which sterigmatocystin was identified, but below the 0.50 ng/g (estimated level = 0.39 ng/g); m/z 325→281 (upper) and m/z 325→310 (lower).

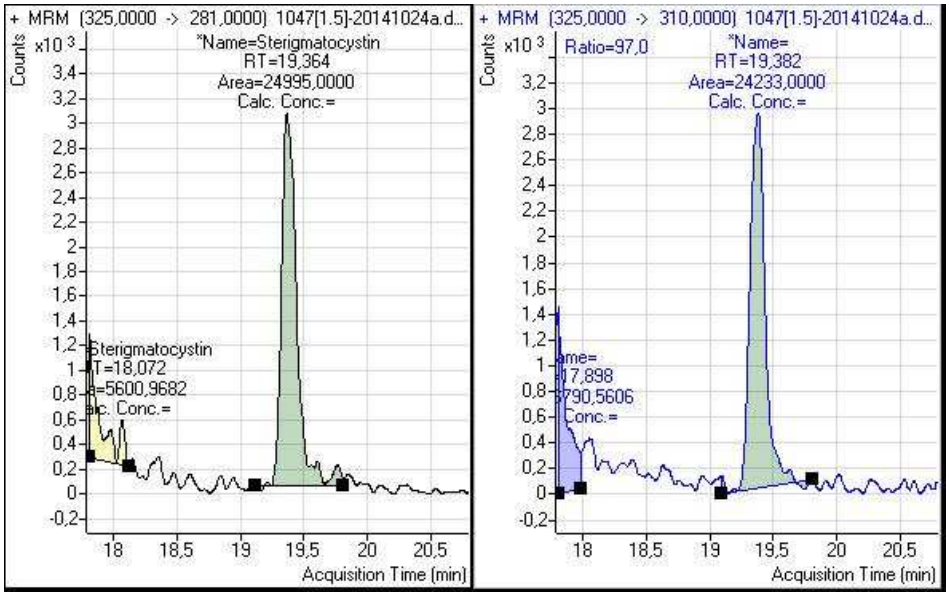
Food and Environment Research agency – Fera, UK



Top: Solvent standard at 1 µg/kg equivalent
Bottom: Sample STER456 S14-052405 Quantified at 1.26 µg/kg
S14-052405 Soft Wheat (NABIM Group 3)

Benaki Phytopatological Institute – BPI, Greece





Solvent standard at 0.094 µg/mL.
Rye grain sample containing 0.75 µg/kg

ABBREVIATIONS

APCI	Atmospheric Pressure Chemical Ionisation
BPI	Benaki Phytopathological Institute
EEA	European Economic Area
EFSA	European Food Safety Authority
ESI	Electro Spray Ionisation
Fera	Food and Environment Research Agency
g	gram
HPLC	High performance liquid chromatography
kg	Kilogram
L	Litre
LB	Lower bound
LC	Liquid chromatography
LC-MS/MS	Liquid chromatography with tandem mass spectrometry
LOD	Limit of detection
LOQ	Limit of quantification
MB	Middle bound
mL	Millilitre
mm	Millimetre
MOE	Margin of exposure
MS	Mass spectrometry
m/z	mass over charge ratio
ng	nanogram
NVWA	Netherlands Food and Consumer Product Safety Authority
PBS	Phosphate buffered saline
PTFE	Polytetrafluoroethylene
RSD_r	Relative standard deviation under repeatability conditions
RSD_R	Relative standard deviation under reproducibility conditions
S/N	Signal-to-noise (ratio)
UB	Upper bound
UCSC	Università Cattolica del Sacro Cuore
µg	microgram
UHPLC	Ultra high performance liquid chromatography
UK	United Kingdom