Diet Study (TDS) – Mycotoxin Analysis

Final report

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Fera Science Ltd, York (UK)
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1. Executive Summary

A total diet study (TDS) is representative of the whole diet. A TDS is different from many surveys in that foods are prepared for consumption (rather than being analysed as sold) before being pooled into groups before analysis. For this TDS, samples for 138 categories of foods established by the Food Standards Agency (FSA) were purchased from 24 local authorities (a total of 3312 samples). The categories were classified under twenty eight food groups and seventeen of these were analysed in the mycotoxin study. These included Bread, Miscellaneous Cereals, Offal, Oils and Fats, Eggs, Sugars and Preserves, Potatoes, Other Vegetables, Fresh Fruit, Fruit Products, Non-alcoholic Beverages, Milk, Dairy Products, Nuts, Alcoholic Drinks, Snacks and Sandwiches.

The samples were analysed for a range of mycotoxins; aflatoxins, ochratoxin A, fumonisins, patulin, zearalenone, trichothecenes, sterigmatocystin, ergot alkaloids, citrinin, cyclopiazonic acid and moniliformin. The 17 food group samples were also analysed for mycotoxins to compare with mathematical calculations carried out on the results for the food categories to check the homogeneity of the food groups.

The most frequently detected toxins were deoxynivalenol and ergot alkaloids which were detected in all bread samples and sandwiches as well as other cereal products. None of the samples exceeded any maximum permitted limit. Very few residues of any of the other mycotoxins analysed were found in the samples tested, most results were below the limits of quantification which were as low as technically achievable, and typically in the sub or low µg/kg range.

This is the first UK TDS study for mycotoxins. These results show very little incidence of mycotoxins in UK food samples, with very few results above the low limits of quantification. The data can be used for future intake calculations to calculate background exposure to various mycotoxins from the whole diet and also to compare exposures to those calculated by other sources.
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### 3. Glossary

<table>
<thead>
<tr>
<th>Acronym</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>EMAN</td>
<td>European Mycotoxin Awareness Network</td>
</tr>
<tr>
<td>FB$_1$</td>
<td>Fumonisin B$_1$</td>
</tr>
<tr>
<td>FB$_2$</td>
<td>Fumonisin B$_2$</td>
</tr>
<tr>
<td>FB$_3$</td>
<td>Fumonisin B$_3$</td>
</tr>
<tr>
<td>Fera</td>
<td>Fera Science Ltd.</td>
</tr>
<tr>
<td>FSA</td>
<td>Food Standards Agency (UK)</td>
</tr>
<tr>
<td>HILIC</td>
<td>Hydrophobic Interaction Liquid Chromatography</td>
</tr>
<tr>
<td>HPLC</td>
<td>High Performance Liquid Chromatography</td>
</tr>
<tr>
<td>IAC</td>
<td>Immunoaffinity Column</td>
</tr>
<tr>
<td>LC-MS/MS</td>
<td>Liquid Chromatography tandem Mass Spectrometry</td>
</tr>
<tr>
<td>LOD</td>
<td>Limit of Detection</td>
</tr>
<tr>
<td>LOQ</td>
<td>Limit of Quantification</td>
</tr>
<tr>
<td>MRM</td>
<td>Multiple Reaction Monitoring</td>
</tr>
<tr>
<td>m/z</td>
<td>Mass to charge ratio</td>
</tr>
<tr>
<td>PBS</td>
<td>Phosphate Buffered Saline</td>
</tr>
<tr>
<td>QC</td>
<td>Quality Control</td>
</tr>
<tr>
<td>RSD</td>
<td>Relative Standard Deviation</td>
</tr>
<tr>
<td>RSD$_r$</td>
<td>Repeatability</td>
</tr>
<tr>
<td>s : n</td>
<td>Signal to noise ratio</td>
</tr>
<tr>
<td>SOP</td>
<td>Standard Operating Procedure</td>
</tr>
<tr>
<td>TDS</td>
<td>Total Diet Study</td>
</tr>
<tr>
<td>UPLC-MS/MS</td>
<td>Ultra Performance Liquid Chromatography tandem Mass Spectrometry</td>
</tr>
</tbody>
</table>
4. Introduction

The key principle of a total diet study (TDS) is that it is representative of the whole diet. A TDS is different from many surveys in that foods are prepared for consumption (rather than being analysed as sold) before being pooled into groups before analyses. For this TDS, samples for each of the 138 categories of foods established by the FSA were purchased from 24 local authorities (a total of 3312 samples). Categories were grouped under twenty eight food groups so that commodities known to be susceptible to contamination (e.g. offal, fish) are kept separate, as are foods which are consumed in large quantities (e.g. bread, potatoes, milk). The relative proportion of foodstuffs in categories within a group (i.e. the amount of food in each category making up the pooled group sample) reflected its importance in the average UK household diet. This is based on three previous years of food purchase data from the Family Food Survey (previously the National Food Survey). The data from the family food survey is purchase data and not consumption data.

A TDS on the levels of metals and other elements has been carried out for the FSA (Fera FD Report 15/06). The samples collected for this main study were also used to measure the levels of mycotoxins in order to:

- calculate background exposure to various mycotoxins from the whole diet
- compare exposures to those calculated by other sources

The 28 food groups in the general TDS are Bread, Miscellaneous Cereals, Carcase Meat, Offal, Meat Products, Poultry, Fish, Oils and Fats, Eggs, Sugars and Preserves, Green Vegetables, Potatoes, Other Vegetables, Canned or Jarred Vegetables, Fresh Fruit, Fruit Products, Non-alcoholic Beverages, Milk, Dairy Products, Nuts, Alcoholic Drinks, Meat Substitutes, Snacks, Desserts Sandwiches, Condiments, Tap Water and Bottled water.

In the mycotoxin TDS, 17 of the 28 food groups which are known to have mycotoxin contamination were studied. These were Bread, Miscellaneous Cereals, Offal, Oils and Fats, Eggs, Sugars and Preserves, Potatoes, Other Vegetables, Fresh Fruit, Fruit Products, Non-alcoholic Beverages, Milk, Dairy Products, Nuts, Alcoholic Drinks, Snacks and Sandwiches.
5. Aims and Objectives

5.1. Scope

Samples prepared for the TDS on metals and other elements were used for this study. However not all mycotoxins were tested for in every food group and category.

The food groups included in the mycotoxin study are listed below:

- Group 1 - Bread
- Group 2 - Miscellaneous Cereals
- Group 4 - Offal
- Group 8 - Oils and Fats
- Group 9 - Eggs
- Group 10 - Sugars and Preserves
- Group 12 - Potatoes
- Group 13 - Other Vegetables
- Group 15 - Fresh Fruit
- Group 16 - Fruit Products
- Group 17 - Beverages (With Mineral Water)
- Group 18 - Milk
- Group 19 - Dairy Products
- Group 20 - Nuts
- Group 21 - Alcoholic drinks
- Group 23 - Snacks
- Group 25 - Sandwiches

The categories that comprised each food group are shown in Table 1.

For consistency with previous total diet studies, the appropriate quantities of the homogenate from the 138 categories were combined into the respective food groups for analyses.

One of the problems with pooling the categories into food groups straight away is that contaminants are often diluted to the extent that they cannot be detected by the analytical method. As well as analysing the individual category samples, the food
groups were also analysed to compare with mathematical calculations carried out on the results for the 138 food categories to check the homogeneity of the food groups tested.

Table 1. Food groups and categories

<table>
<thead>
<tr>
<th>Group</th>
<th>Category</th>
<th>Category</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Bread</td>
<td>Fresh fruit</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
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<td>3</td>
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<td></td>
<td>7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>8</td>
</tr>
<tr>
<td>2</td>
<td>Miscellaneous cereals</td>
<td>Fruit products</td>
</tr>
<tr>
<td>3</td>
<td>Offals</td>
<td>Non-alcoholic Beverages (with bottled water)</td>
</tr>
<tr>
<td>4</td>
<td>Oils and fats</td>
<td>Milk</td>
</tr>
<tr>
<td>5</td>
<td>Eggs</td>
<td>Dairy products</td>
</tr>
<tr>
<td>6</td>
<td>Sugars and preserves</td>
<td>Nuts</td>
</tr>
<tr>
<td>7</td>
<td>Potatoes</td>
<td>Alcoholic drinks</td>
</tr>
<tr>
<td>8</td>
<td>Other vegetables</td>
<td>Snacks</td>
</tr>
<tr>
<td>9</td>
<td></td>
<td>Sandwiches</td>
</tr>
</tbody>
</table>

The intention was that this approach would:

- provide specific data on mycotoxin concentrations at the food category level and food group level, rather than just at the food group level
- allow the FSA to refine exposure assessments for each food category
- allow the FSA to carry out exposure assessments for high consumers of specific foods
• help identify any ‘hotspots’ in particular food categories.

The samples were analysed for a range of mycotoxins; aflatoxins (B$_1$, B$_2$, G$_1$, G$_2$ and M$_1$), ochratoxin A, fumonisins (B$_1$, B$_2$ and B$_3$), patulin, zearalenone, trichothecenes (deoxynivalenol, 3-acetyldeoxynivalenol, 15-acetyldideoxynivalenol, nivalenol, fusarenon-X, diacetoxyisocirpenol, neosolaniol, HT-2 toxin and T-2 toxin), sterigmatocystin, ergot alkaloids (ergocornine, ergocorninine, ergocristine, ergocristinine, ergocryptine, ergocryptinine, ergometrine, ergometrinine, ergosine, ergosinine, ergotamine and ergotaminine), citrinin, cyclopiazonic acid and moniliformin.

5.2. Sampling

Sampling was conducted by HallMark, contracted by the FSA. Samples were purchased throughout the UK and prepared according to a protocol agreed with the FSA. The protocol was intended to be suitable for metals/element analysis and was not designed for mycotoxin analysis.

5.3. Analysis

The samples were extracted using solvent and the cleaned-up extracts were analysed using the method that would allow the maximum sensitivity (lowest limit of quantification). In many cases, state of the art LC-MS/MS was used due to the high sensitivity and selectivity of the instrumentation, however HPLC-fluorescence was also used for some analyses where method performance was already well established or better sensitivity could be achieved.

In total 615 separate mycotoxin determinations were requested, and 634 results are reported.

5.4. Method Development and Validation

Many methods used were UKAS accredited. Where required, improved extraction and instrumental methods were developed. Validation samples were analysed to establish method repeatability and recovery but full single laboratory validation was not undertaken as there was insufficient time to do this for all the methods within the time constraints of this project.

5.5. Quality Control

Fully established UKAS accredited methods were used for this study where they were available. Blank samples and spikes were included. In some cases, the scope of these accredited methods did not cover all the matrices included in this study. In the cases where non-accredited methods were used quality control (QC) was increased by overspiking all test samples or by the inclusion of an isotopically labelled internal standard. For accredited methods, samples were batched into similar products with recovery adjustment being made using recovery determined for
the product type that was the closest match to the sample, or the group sample recovery was used to adjust for all the category samples that made up the group.

5.6. Reporting of Results

Results have been assessed and the recovery value calculated for individual samples, or group or close equivalent sample type. Each sample has been assessed and an individual limit of quantification (LOQ) calculated. This takes into account the apparent recovery from a spiked sample, therefore samples with a lower recovery will have a higher apparent LOQ than might be suggested by measuring signal to noise (s : n) from solvent calibration standards. This is a ‘truer’ representation of what could be measured in each sample type. All results are reported corrected for recovery and are in µg/kg.

6. Materials and Methods

6.1. Sampling

Sampling was conducted by another contractor appointed by the FSA. Details of the sampling plan and delivery schedule are in the report of the metals analysis of TDS samples. Samples were delivered to Fera in batches.

Samples were stored under suitable dry conditions, frozen if necessary, before being prepared and homogenised.

6.2. Sample Preparation

Sample preparation was carried out according to a previously agreed protocol for metals analysis (Metals TDS report FD Report 15/06). All samples were stored at -18°C after preparation until analysis.

6.3. Chemicals and Reagents

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.

To compensate for matrix effects during LC-MS/MS analysis, carbon-13 (13C) isotopically labelled internal standards were used. The internal standards:

- U-[13C18]-Sterigmatocystin in acetonitrile
- U-[13C13]-Citrinin in acetonitrile
- U-[13C20]-Cyclopiazonic acid in acetonitrile
U-[\textsuperscript{13}C\textsubscript{15}]\text{-Deoxynivalenol in acetonitrile
\[ \text{U-[\textsuperscript{13}C\textsubscript{7}] Patulin in acetonitrile} \]

were purchased from Romerlabs, but were sourced from Biopure (Tulln, Austria).
The analytical reference standards of sterigmatocystin, ochratoxin A, patulin,
aflatoxins B\textsubscript{1}, B\textsubscript{2}, G\textsubscript{1}, G\textsubscript{2} and M\textsubscript{1} and zearalenone were purchased as solid (dry film, Sigma Chemicals, \(\geq 98\%\) purity). The analytical reference standards deoxynivalenol, 3-acetyldeoxynivalenol, 15-acetyldeoxynivalenol, nivalenol, fusarenon-X, diacetoxyscirpenol, neosolaniol, T-2 toxin, HT-2 toxin, citrinin, cyclopiazonic acid, moniliformin, fumonisins B\textsubscript{1}, B\textsubscript{2} and B\textsubscript{3} were purchased as solutions in acetonitrile from Romerlabs, but were sourced from Biopure (Tulln, Austria). Ergot alkaloids (6 ine plus inine forms) were produced at IFA, Tulln and were gifted to Fera; further ergot alkaloids standards were purchased via Romerlabs from Biopure (Tulln, Austria).

Immunoaffinity columns for ochratoxin A, aflatoxins, zearalenone and fumonisins and Mycosep clean-up columns for trichothecenes were purchased from R-Biopharm (Rhone). Prototype citrinin immunoaffinity columns were a gift from R-Biopharm (Rhone). Dispersive SPE material (Bondesil PSA, 40 \(\mu\)m) was from Varian. Syringe filters (0.22 \(\mu\)m nylon, 13 mm) were from Anachem.

### 6.4. Extraction and Clean-Up

Targeted methods were used for each toxin or group of toxins however, where possible, common extraction and clean-up strategies were employed. In all cases the sample was extracted with organic solvent and water. This was followed by either a filtration or centrifugation step. The extract was diluted and cleaned-up or, for some LC-MS/MS methods, diluted and analysed directly (‘dilute and shoot’).

Isotopically labelled (carbon-13) standards were used to internally standardise and adjust for matrix effects for LC-MS/MS methods where they were available.

UKAS accredited procedures for Quality Assurance, calibration and calculation of results were used.

Where LC-MS/MS was used the accredited SOP for LC-MS analysis was followed. This stipulates criteria for ion ratio and retention time acceptance following the criteria outlined in SANCO/12571/2013 (Pesticides criteria document).

Further details of the methods used are given below.

### 6.5. LC-MS/MS analysis

For all LC-MS/MS methods the following conditions and equipment were used:

<table>
<thead>
<tr>
<th>Chromatograph:</th>
<th>Waters Acquity UPLC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Autosampler:</td>
<td>Waters Acquity UPLC</td>
</tr>
</tbody>
</table>
Weak needle wash: 80:20 (v/v) water : methanol
Strong needle wash: 20:40:40 (v/v/v) water : methanol : acetonitrile
Mass spectrometer: Waters Xevo TQ-S triple quadrupole
Desolvation temperature: 500°C
Desolvation gas flow rate: 1000 L/h
Nebuliser gas flow: 7 bar
Source temperature: 150 C
Cone gas flow rate: 100 L/h
Resolution: Unit mass

6.5.1. UPLC Gradient “Curve”

In the tables for each method below a gradient “curve” is given. This is the gradient profile defined by the instrument software and are defined as follows.

Curve 6 is a linear change from one condition to the next.

Curve 1 is a step change in conditions at the start of the time segment.

Curve 5 is a variable rate of change from one condition to the next, with the rate of change slightly more rapid at the start of the time segment than at the end.

6.5.2. Data Analysis and Quantification

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. Where available a 13C-labelled internal standard was used to internally standardise the method, and inherently correct analytical results.

7. UKAS Accredited Methods

UKAS accredited protocols for the extraction and clean-up of samples for aflatoxins, ochratoxin A, fumonisins, patulin, zearalenone and trichothecenes were used, following the extraction procedure validated for the matrix under test, or for a similar matrix. The methods for ergot alkaloids and sterigmatocystin are also being submitted for UKAS accreditation under Extension of Scope.
7.1. Aflatoxins / Ochratoxin A

7.1.1. Extraction

Samples were extracted with a mixture of organic solvent and water, or sodium hydrogen bicarbonate solution depending on the matrix, using protocols from in-house methods FSG 251, FSG 252 and FSG 261. Following dilution with phosphate buffered saline (PBS) extracts were cleaned up by immunoaffinity column, using either Afla/Ochra columns where both analytes were required, or Aflaprep, or Ochraprep columns where only one analyte was requested. Most samples were spiked at 5 µg/kg each aflatoxin and ochratoxin A, alcoholic beverages were spiked at 2 µg/kg. Where the method was not accredited for the matrix, each sample was overspiked.

Samples in Group 19 Dairy products were analysed for aflatoxin M₁, using in-house method FSG 300, and were cleaned up with Aflaprep columns. Milk samples and samples 116 and 117 (condensed milk and instant milk) were analysed using in house method FSG 253.

7.1.2. HPLC Analysis

Cleaned up extracts were analysed by reversed phase HPLC with fluorescence detection, either using a method for aflatoxins B₁, B₂, G₁ and G₂, or ochratoxin A, or a multi method capable of detecting all compounds. Post-column derivatisation using a KOBRA cell was used to derivatise aflatoxin B₁ and G₁. The HPLC pump to deliver mobile phase was set at 1.0 mL/min, a Gilson automatic sample processor and injector (or equivalent) and a Fluorescence detector with programmable functions (Jasco FP1520 or similar) were used. Calibration standards in the range equivalent to 1 to 5 µg/kg for each toxin were used.

The HPLC column was a Spherisorb ODS1-Excel (25 cm x 4.6 mm i.d.), 250 Å pore size, 5 µm spherical particles (or equivalent).

Mobile Phase A: Acetonitrile: methanol: 0.1 % orthophosphoric acid solution (24:24:52, v/v/v).

Mobile Phase B: Acetonitrile: methanol: 0.1 % orthophosphoric acid solution (14.4:54.4:31.2, v/v/v).

The gradient profile used for joint aflatoxin/ochratoxin A analysis is given in Table 2.
Table 2. Gradient profile for joint aflatoxin / ochratoxin A method.

<table>
<thead>
<tr>
<th>Time</th>
<th>Mobile phase A</th>
<th>Mobile phase B</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 to 17 minutes</td>
<td>100 %</td>
<td>0 %</td>
</tr>
<tr>
<td>19 to 29 minutes</td>
<td>0 %</td>
<td>100 %</td>
</tr>
<tr>
<td>31 to 40 minutes</td>
<td>100 %</td>
<td>0 %</td>
</tr>
</tbody>
</table>

Aflatoxin M₁ samples were analysed by HPLC with fluorescence detection. The HPLC column was a Spherisorb ODS2-Excel (25 cm x 4.6 mm i.d.), 5 µm particle size, 250 Å pore size (or equivalent) with guard column (C₁₈, 2.5 cm x 4.6 mm i.d.). Mobile phase of water : methanol : acetonitrile (60 : 10 : 30, v/v/v) was pumped at 1 mL/min. Calibration standards in the range equivalent to 0.02 to 0.1 µg/kg were used.

7.1.3. HPLC Detection

For aflatoxin / ochratoxin A the programmable fluorescence detector was set to the following parameters:

Excitation = 364 nm, Emission = 440 nm from 0 to 18 min

Excitation = 333 nm, Emission = 477 nm from 18 min onwards

For aflatoxin M₁ the fluorescence detector was set at:

Excitation = 364 nm, Emission = 434 nm.

7.2. Zearalenone

7.2.1. Extraction

Analysis was carried out following in-house method FSG 258. Sample (12.5 g) was weighed into a plastic beaker, and extraction solution of acetonitrile : water (75 : 25, v/v) added. Samples were homogenised for 3 minutes with an Ultra Turrax set at high speed, then filtered through Whatman No. 4 or Whatman 113V (fluted filter). An aliquot (12 mL) of filtrate was diluted with 88 mL of PBS in a conical flask. The diluted filtrate was transferred to a pre-labelled tube for automated immunoaffinity column clean-up using a ZONprep IAC.

For oil, 2.0 g oil was weighed into a centrifuge tube and 2 mL hexane and 20 mL methanol/ammonium carbonate solution added. This was vortex mixed then shaken for 15 minutes. The extracts were centrifuged at 4000 rpm, 4 °C for 10 minutes and then 10 mL of the upper methanol-water layer removed and adjusted to pH 6 to ≤ 7.5. An aliquot (5 mL) of the extract was evaporated to dryness under nitrogen, and redissolved in 1 mL HPLC mobile phase. The extract was analysed by HPLC.
Samples were spiked at a level equivalent to 50 µg/kg. For accredited matrices samples were grouped and a representative sample spiked, for non-accredited matrices each sample was overspiked.

7.2.2. HPLC Analysis

Clean-up and analysis were carried out automatically by a Gilson ASPEC system (except for oils). The HPLC column was a Spherisorb ODS2-Excel (25 cm x 4.6 mm i.d.), 5 µm particle size, 250 Å pore size or equivalent, with a mobile phase of water : acetonitrile (45 : 50 v/v) pumped at 1 mL/min. Detection was by fluorescence with excitation and emission wavelengths of 275 and 450 nm. Calibration range was equivalent to 2.5 to 50 µg/kg.

7.3. Fumonisins

7.3.1. Extraction

Samples were extracted following in-house method FSG 264. Sample (12.5 g) was weighed into a beaker. Extraction solvent (62.5 mL, water : acetonitrile : methanol, 50 : 25 : 25, v/v/v) was added and the sample homogenised using an Ultra Turrax blender for approximately 5 minutes. The sample was filtered through 113V filter paper, then an aliquot (10 mL) transferred into a flask containing 40 mL of PBS. This was mixed then filtered through microfibre filter paper.

For oil, 12.5 g was measured into a separating funnel and shaken with 62 mL of extraction solvent (0.1 M o-phosphoric acid solution : acetonitrile : methanol, 50 : 25 : 25, v/v/v). The bottom, aqueous, layer was drained into a flask, 10 mL was diluted with 40 mL PBS and filtered through microfibre filter paper.

Samples were cleaned-up by immunoaffinity column. Fumonisins were eluted with 1.5 mL of methanol followed by 1.5 mL of water. An aliquot was filtered through a 0.22 µm syringe filter and transfer to a vial for LC-MS/MS analysis. Spiking level was at 250/171/125 µg/kg FB1/FB2/FB3 and 100/50/50 µg/kg FB1/FB2/FB3 for the two batches.

7.3.2. LC-MS/MS Analysis

Cleaned-up extracts were analysed by UPLC-MS/MS. Fumonisins B1, B2 and B3 were included in the method. The column was a Waters Acquity HSS T3 1.8 µm (100 x 2.1 mm) at a column temperature of 40 °C. The injection volume was 2 µL. The following UPLC gradient was used:

Mobile phase A: 0.1 % formic acid in water

Mobile phase B: 0.1 % formic acid in 1:1 (v/v) methanol : acetonitrile

The gradient profile used is given in Table 3. MRM transitions are given in Table 4.
Table 3. Gradient profile for Fumonisin analysis.

<table>
<thead>
<tr>
<th>Time / min</th>
<th>% B</th>
<th>Flow rate / mL/min</th>
<th>Curve</th>
</tr>
</thead>
<tbody>
<tr>
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</tr>
<tr>
<td>4.5</td>
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<td>0.4</td>
<td>6</td>
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</table>

Table 4. MRM Transitions for Fumonisin analysis.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Parent</th>
<th>Fragment</th>
<th>Cone Voltage / V</th>
<th>Collision Energy / eV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fumonisin B₁</td>
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<td>37</td>
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<tr>
<td></td>
<td></td>
<td>352.2</td>
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<td>34</td>
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<td>Fumonisin B₂</td>
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<tr>
<td></td>
<td></td>
<td>318.2</td>
<td></td>
<td>37</td>
</tr>
<tr>
<td>Fumonisin B₃</td>
<td>706.4</td>
<td>336.2</td>
<td>40</td>
<td>34</td>
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<tr>
<td></td>
<td></td>
<td>354.2</td>
<td></td>
<td>32</td>
</tr>
</tbody>
</table>

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 fumonisins. Concentrations corresponding to 40/20/20 to 500/250/250 µg/kg FB₁/FB₂/FB₃ in the sample were used in one batch and 5/2.5/2.5 to 250/125/125 µg/kg used in the second.

7.4. Trichothecenes

The trichothecenes included in the study were deoxynivalenol, 3-acetyl-deoxynivalenol, 15-acetyldeoxynivalenol, nivalenol, fusarenon-X, diacetoxyiscirpenol, neosolaniol, HT-2 toxin and T-2 toxin.

7.4.1. Extraction

10 g of sample was weighed into a beaker, and 50 mL of extraction solvent (acetonitrile : water, 84 : 16, v/v) was added. This was homogenised using an Ultra Turrax blender for approximately 5 minutes. Extracts were filtered through 113V filter paper. An aliquot (6-7 mL) of the filtered extract was transferred into a glass tube supplied with the charcoal/alumina column. The clean-up column was pushed through the extract at a rate of about 1 mL per 30 seconds until approximately 3-4 mL of cleaned up extract has collected above the bed of the clean-up column. 4 mL of cleaned-up filtrate was transferred into a vial. The cleaned-up filtrate was
evaporated to dryness under nitrogen then re-dissolved in methanol : water (20 : 80, v/v) and filtered through a syringe filter prior to LC-MS/MS analysis.

Selected samples were re-analysed to confirm initial results, at which time a $^{13}$C-labelled internal standard for deoxynivalenol was included to account for matrix effects.

7.4.2. LC-MS/MS analysis

Samples were analysed by UPLC-MS/MS. The column was a Restek Raptor Biphenyl 2.7 µm (100 x 2.1 mm) maintained at 40 °C. The injection volume was 5 µL. The following UPLC gradient was used:

- **Mobile phase A:** 1 mM ammonium formate in water
- **Mobile phase B:** 1:1 (v/v) methanol : acetonitrile

The gradient profile used is given in Table 5. Nine compounds were included in the method and the MRM Transitions used are given in Table 6.

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 trichothecenes (concentrations corresponding to 5, 10, 25, 50, 100 and 200 µg/kg in the sample, internal standard at 50 µg/kg). Samples were spiked at 100 µg/kg each trichothecene.

### Table 5. Gradient profile used for trichothecene analysis.

<table>
<thead>
<tr>
<th>Time / min</th>
<th>% B</th>
<th>Flow rate / mL/min</th>
<th>Curve</th>
</tr>
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<td>6</td>
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Table 6. MRM Transitions used for trichothecene analysis.

<table>
<thead>
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<th>Compound</th>
<th>Parent</th>
<th>Fragment</th>
<th>Cone Voltage / V</th>
<th>Collision Energy / eV</th>
</tr>
</thead>
<tbody>
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<td>249.1</td>
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<tr>
<td></td>
<td></td>
<td>231.1</td>
<td></td>
<td>12</td>
</tr>
<tr>
<td>13C15-Deoxynivalenol</td>
<td>312.1</td>
<td>263.1</td>
<td>20</td>
<td>10</td>
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<tr>
<td></td>
<td></td>
<td>245.1</td>
<td></td>
<td>12</td>
</tr>
<tr>
<td>Nivalenol</td>
<td>313.1</td>
<td>175.0</td>
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<tr>
<td></td>
<td></td>
<td>125.0</td>
<td></td>
<td>12</td>
</tr>
<tr>
<td>3- and 15-Acetyl-Deoxynivalenol</td>
<td>339.1</td>
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<tr>
<td></td>
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<td>Fusarenon X</td>
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<td>229.0</td>
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<td>15</td>
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<td>Diacetoxyscirpenol</td>
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<td></td>
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<td>Neosolaniol</td>
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<td>215.1</td>
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<td>HT-2 toxin</td>
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<td></td>
<td></td>
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<td>12</td>
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<tr>
<td>T-2 toxin</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>215.1</td>
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<td>18</td>
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</tbody>
</table>

7.5. Ergot Alkaloids

The ergot alkaloids included in the study were ergocornine, ergocorninine, ergocristine, ergocristinine, ergocryptine, ergocryptinine, ergometrine, ergometrinine, ergosine, ergosinine, ergotamine and ergotaminine.

7.5.1. Extraction

The method used for ergot alkaloids has been validated and published (Krska et al, 2008). In-house method FSG 601 was used. Samples (5 g) were extracted with a mixture of acetonitrile : ammonium carbonate solution (84:16, v/v) on an orbital shaker for 30 minutes. After shaking the sample extracts were filtered. 1 mL of sample was removed, and transferred into a 4 mL amber glass vial containing 50 mg of Varian Bondesil solid phase material. Samples were vortex mixed then an aliquot filtered through a 13 mm PTFE 0.22 µm filter into a 2 mL amber vial and transferred to a 200 µL vial ready for UPLC-MS/MS.

7.5.2. LC-MS/MS Analysis

Samples were analysed using a modified version of the published LC-MS/MS method. The method was capable of detecting all 6 major ergot alkaloids and their -inine epimers and used alkaline mobile phase conditions to prevent unwanted epimerisation. The column was a Waters Acquity BEH C18 1.7 µm (100 x 2.1 mm) at 40 °C. The injection volume was 2 µL. The mobile phase and gradient profile were as follows:
Mobile phase A: 200 mg/L ammonium carbonate in water

Mobile phase B: Acetonitrile

The gradient profile used is given in Table 7.

Twelve compounds were included in the method and the MRM transitions used are given in Table 8.

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 ergot alkaloids (concentrations corresponding to 1 to 200 µg/kg equivalent in the sample, samples were spiked at 50 µg/kg).

Table 7. Gradient profile used for ergot alkaloid analysis.

<table>
<thead>
<tr>
<th>Time / min</th>
<th>% B</th>
<th>Flow rate / mL/min</th>
<th>Curve</th>
</tr>
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Table 8. MRM transitions used for ergot alkaloid analysis.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Parent</th>
<th>Fragment</th>
<th>Cone Voltage / V</th>
<th>Collision Energy / eV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ergometrine</td>
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<tr>
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<td></td>
<td>208.1</td>
<td></td>
<td>27</td>
</tr>
<tr>
<td>Ergometrinine</td>
<td>326.2</td>
<td>208.1</td>
<td>30</td>
<td>27</td>
</tr>
<tr>
<td></td>
<td></td>
<td>223.1</td>
<td></td>
<td>24</td>
</tr>
<tr>
<td>Ergosine</td>
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<td>223.1</td>
<td>30</td>
<td>30</td>
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<tr>
<td></td>
<td></td>
<td>208.1</td>
<td></td>
<td>40</td>
</tr>
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<td>30</td>
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<td></td>
<td></td>
<td>208.1</td>
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<td>Ergocornine</td>
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<tr>
<td></td>
<td></td>
<td>223.1</td>
<td></td>
<td>35</td>
</tr>
<tr>
<td>Ergocorninine</td>
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<td>544.3</td>
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<td></td>
<td></td>
<td>223.1</td>
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<td>35</td>
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<td></td>
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<td>277.1</td>
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<td>Ergocryptine</td>
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<td>Ergocryptinine</td>
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<td>558.3</td>
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<td></td>
<td>223.1</td>
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<td></td>
<td>305.2</td>
<td></td>
<td>26</td>
</tr>
</tbody>
</table>

7.6. Patulin

7.6.1. Extraction

The extraction procedure in the UKAS accredited in-house method FSG 254 was used with some minor modifications. The method is only accredited for apple juice, other fruit juices and apple puree. Sample (10 g) was weighed, and extracted with ethyl acetate. The ethyl acetate was washed with aqueous sodium hydrogen carbonate solution which was discarded. Acetic acid was added to the ethyl acetate and this was evaporated to dryness and extracts redissolved in water adjusted to pH 4 by addition of acetic acid.

For fruit and vegetable samples the method for apple puree was followed. A portion of sample was weighed, mixed with water and incubated with pectinase enzyme. After centrifugation the supernatant was extracted with ethyl acetate as above. 13C-labelled patulin internal standard was added to all samples prior to extraction.

7.6.2. LC-MS/MS Analysis

Samples were analysed by UPLC-MS/MS. The column was a Waters Acquity HSS T3 1.8 µm (100 x 2.1 mm), maintained at 40 °C, with mobile-phase gradient of 1 mM ammonium formate in water and methanol : acetonitrile (50:50 v/v) from 99:1 (0 min) to 70:30 in 3 min, then to 1:99 in 3 min, and back to 99:1 by 8 min. The injection volume was 5 µL.

For patulin, using [M+H]+ (m/z 153) as precursor ion, two transitions were measured: fragment m/z 109 (35 V) [quantification ion], and m/z 81 (35 V). For the isotopic label (13C7), 160 m/z was used as precursor ion with m/z 115 (35 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 patulin (concentrations corresponding to 2, 5, 10, 25, 40 and 50 µg/kg in the sample, internal standard at 25 µg/kg). 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.

7.7. Sterigmatocystin

7.7.1. Extraction

To 5 g dry milled homogenate 20 mL of acetonitrile : water (80 : 20 v/v) was added and the sample extracted using an orbital shaker for 2 hours. 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 filtered using a 0.22 µm syringe filter into an autosampler vial and analysed by LC-MS/MS.

Isotopically labelled sterigmatocystin in acetonitrile was added (equivalent to 1.5 µg/kg) was added to the sample prior to extraction.

7.7.2. LC-MS/MS Analysis

Sample extract (2 µL) was injected into an UPLC-MS/MS system. Separation was performed on a Waters Acquity HSS T3 1.8 µm (100 x 2.1 mm) 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 min) to 5 : 95 in 3 min, then isocratic for 3.6 min. The flow rate was 0.4 mL/min.

For sterigmatocystin, using [M+H]+ (m/z 325) as precursor ion, two transitions were measured: m/z 310 (23 eV) [quantification ion], and m/z 281 (35 eV). For the isotopic label (13C18), m/z 343 was used as precursor ion with m/z 297 (36 eV) 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). 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.

7.8. Citrinin

7.8.1. Extraction

Samples were extracted with methanol and water, and after filtration the extract was diluted with water adjusted to pH 7.4 and cleaned up by immunoaffinity column. 5 g sample was weighed and 13C-citrinin internal standard added at a level equivalent to 25 µg/kg. 25 mL extraction solvent methanol : water (75 : 25, v/v) was added and samples were mixed by vortex mixing then placed on a shaker for 30 minutes. After extraction samples were filtered through Whatman 113V equivalent filter paper and 2 mL of filtered extract was added to 18 mL PBS. This was mixed well then filtered
through GFA filter paper. Extracts were cleaned up using immunoaffinity columns (RBiopharm Rhone columns). Samples were eluted in 2 mL methanol, then this was evaporated to dryness under nitrogen and redissolved in 1 mL methanol : water (1 : 1, v/v). The sample was transferred to a vial for LC-MS/MS analysis.

7.8.2. LC-MS/MS Analysis

Samples were analysed by LC-MS/MS using acidic mobile phase conditions. The 13C-citrinin standard was used to internally standardise the method and adjust for matrix effects. The column used was a Waters Acquity HSS T3 1.8 µm (100 x 2.1 mm), at 40 °C. The injection volume was 5 µL. The following UPLC gradient profile was used:

Mobile phase A: 0.1 % formic acid in water
Mobile phase B: 0.1 % formic acid in 1 : 1 (v/v) methanol : acetonitrile

The gradient profile used in given in Table 9 and the MRM transitions used are in Table 10.

Table 9. Gradient profile used for citrinin analysis.

<table>
<thead>
<tr>
<th>Time / min</th>
<th>% B</th>
<th>Flow rate / mL/min</th>
<th>Curve</th>
</tr>
</thead>
<tbody>
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<td>-</td>
</tr>
<tr>
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<td>99</td>
<td>0.4</td>
<td>6</td>
</tr>
<tr>
<td>5.6</td>
<td>99</td>
<td>0.6</td>
<td>6</td>
</tr>
<tr>
<td>7.0</td>
<td>99</td>
<td>0.6</td>
<td>6</td>
</tr>
<tr>
<td>7.1</td>
<td>15</td>
<td>0.4</td>
<td>6</td>
</tr>
<tr>
<td>9.0</td>
<td>15</td>
<td>0.4</td>
<td>6</td>
</tr>
</tbody>
</table>

Table 10. MRM Transitions for citrinin analysis.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Parent</th>
<th>Fragment</th>
<th>Cone Voltage / V</th>
<th>Collision Energy / eV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Citrinin</td>
<td>251.1</td>
<td>233.1</td>
<td>25</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>251.1</td>
<td>205.1</td>
<td></td>
<td>26</td>
</tr>
<tr>
<td></td>
<td>251.1</td>
<td>91.0</td>
<td></td>
<td>40</td>
</tr>
<tr>
<td>13C13-Citrinin</td>
<td>264.1</td>
<td>246.1</td>
<td>25</td>
<td>16</td>
</tr>
</tbody>
</table>

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 citrinin (concentrations
corresponding to 1, 2.5, 5, 10, 25, and 50 µg/kg in the sample, internal standard at 25 µg/kg).

7.9. Cyclopiazonic acid and Moniliformin

7.9.1. Extraction

Samples were extracted using a common extraction solvent of acetonitrile : water : acetic acid (79 : 20 : 1, v/v/v). 20 mL of extraction solvent was added to 5 g sample and samples were shaken for 2 hours. Carbon-13 labelled cyclopiazonic acid was added to all samples before extraction at 25 µg/kg. After extraction samples were centrifuged, diluted with an equal volume of acetonitrile : water : acetic acid (20 : 79 : 1), then filtered prior to analysis by LC-MS/MS using separate methods for each analyte.

7.9.2. Cyclopiazonic Acid – LC-MS/MS Analysis

Samples were analysed by LC-MS/MS using an alkaline mobile phase. Carbon-13 labelled cyclopiazonic acid was used to internally standardise the method and compensate for matrix effects. The column used was a Waters Acquity BEH C\textsubscript{18} 1.7 µm (100 x 2.1 mm), at 40 °C. The injection volume was 3 µL. The following UPLC gradient profile was used:

Mobile phase A: 200 mg/L ammonium carbonate

Mobile phase B: 1:1 (v/v) methanol : acetonitrile

The gradient profile used is given in Table 11 and the MRM transitions used are given in Table 12.

Table 11. Gradient profile used for cyclopiazonic acid analysis.

<table>
<thead>
<tr>
<th>Time / min</th>
<th>% B</th>
<th>Flow rate / mL/min</th>
<th>Curve</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>10</td>
<td>0.4</td>
<td>-</td>
</tr>
<tr>
<td>4.0</td>
<td>50</td>
<td>0.4</td>
<td>6</td>
</tr>
<tr>
<td>4.5</td>
<td>99</td>
<td>0.4</td>
<td>6</td>
</tr>
<tr>
<td>5.0</td>
<td>99</td>
<td>0.6</td>
<td>6</td>
</tr>
<tr>
<td>7.0</td>
<td>99</td>
<td>0.6</td>
<td>6</td>
</tr>
<tr>
<td>8.9</td>
<td>10</td>
<td>0.6</td>
<td>1</td>
</tr>
<tr>
<td>9.0</td>
<td>10</td>
<td>0.4</td>
<td>6</td>
</tr>
</tbody>
</table>
Table 12. MRM Transitions used for cyclopiazonic acid analysis.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Parent</th>
<th>Fragment</th>
<th>Cone Voltage / V</th>
<th>Collision Energy / eV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cyclopiazonic acid</td>
<td>337.2</td>
<td>196</td>
<td>30</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td></td>
<td>182</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$^{13}$C$_2$O$_2$-Cyclopiazonic acid</td>
<td>357.2</td>
<td>210</td>
<td>30</td>
<td>22</td>
</tr>
</tbody>
</table>

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 cyclopiazonic acid (concentrations corresponding to 1, 2.5, 5, 10, 25, and 50 µg/kg in the sample, internal standard at 25 µg/kg).

7.9.3. Moniliformin– LC-MS/MS Analysis

Samples were analysed by LC-MS/MS using HILIC chromatography. The column used was a SeQuant ZIC-HILIC 5 µm 200 Å (150 x 2.1 mm), with a column filter (Phenomenex KrudKatcher 0.5 µm). The column temperature was 40 °C and the injection volume was 5 µL. The following gradient profile was used:

Mobile phase A: 50 mM ammonium formate in water
Mobile phase B: Acetonitrile

The gradient profile used is given in Table 13. It was only possible to determine one transition for moniliformin, the MRM transition information is given in Table 14.

Table 13. Gradient profile for moniliformin analysis.

<table>
<thead>
<tr>
<th>Time / min</th>
<th>% B</th>
<th>Flow rate / mL/min</th>
<th>Curve</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>95</td>
<td>0.2</td>
<td>-</td>
</tr>
<tr>
<td>5.0</td>
<td>50</td>
<td>0.2</td>
<td>6</td>
</tr>
<tr>
<td>8.0</td>
<td>50</td>
<td>0.2</td>
<td>6</td>
</tr>
<tr>
<td>8.1</td>
<td>95</td>
<td>0.2</td>
<td>6</td>
</tr>
<tr>
<td>23.0</td>
<td>95</td>
<td>0.2</td>
<td>6</td>
</tr>
</tbody>
</table>
Table 14. MRM Transition for moniliformin analysis.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Parent</th>
<th>Fragment</th>
<th>Cone Voltage / V</th>
<th>Collision Energy / eV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moniliformin</td>
<td>97.0</td>
<td>41.0</td>
<td>35</td>
<td>12</td>
</tr>
</tbody>
</table>

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 moniliformin (concentrations corresponding to 1, 2.5, 5, 10, 25, and 50 µg/kg in the sample, samples were overspiked with moniliformin at a level of 25 µg/kg prior to extraction).

7.10. Validation Analyses

Six spiked samples were used to establish recovery and repeatability for citrinin, cyclopiazonic acid and moniliformin. The validation was performed by spiking six replicates of a cereal sample at 25 µg/kg. Samples used in the validation were also analysed without spiking, and where internal standard was used, a double blank with no internal standard was also analysed.

The linearity of the LC-MS/MS measurement was established through calibration standards in solvent, covering the relevant concentration range. From these initial in-house validations, the linearity, recovery, repeatability, selectivity, LOQ and limit of detection (LOD) were derived. In addition, the stability of retention time and ion ratios in solvent standards and extracts were determined.

The LOD is defined here as the level corresponding to a signal-to-noise ratio (s : n) of three.

Identification of analytes was based on retention time and ion ratio of coinciding peaks for at least two diagnostic transitions in the correct abundance ratio. The LC-MS SOP states that Retention Time should be within 2.5 % of the mean of the standards. The ion ratio of the two diagnostic ions (least abundant/most abundant) in the samples should be consistent with that obtained during validation and not deviate more than ± 20 %.

7.11. Quality Control

With each batch of survey samples, one or more spiked samples were included to assess method performance and recovery for different commodities. In many analyses every sample was spiked with the compound of interest and/or the isotopically labelled internal standard where it was used. For some analyses samples were grouped into similar types and spikes were made into one sample chosen to be representative of the group.
7.12. Reporting of Results

Where labelled internal standard was added to the sample before extraction recovery correction was inherent to the procedure.

For samples spiked with parent compound the recovery for each sample or group of samples was calculated and used to correct the results.

In the majority of cases the LOQ was set at level equivalent to the bottom calibrations standard (as stated in the LC-MS/MS SOP), as long as the s : n was good enough (>10 : 1). Some LOQs could be lower based on s : n. LOD was determined by s : n but was also influenced by carry-over and background peaks. Limits of quantification were calculated from signal to noise, and were adjusted for the recovery measured. Results were expressed as a numerical value corrected for recovery where the residue was above the LOQ. Where a residue was seen below the LOQ but above the LOD the result was reported as less than LOQ (<LOQ) and the value found and the LOQ given. Where no residue was detected the result was reported as <LOD, and the calculated (corrected) LOD given.

8. Results

8.1. Existing Mycotoxins Methods

Various procedures were already established in-house for the extraction of different mycotoxins from samples. In many cases it was possible to apply these methods without deviation from the SOP, however the diverse range of sample matrices resulted in the need for adaptations to the extraction procedures.

A generic extraction procedure has also been established for a broad range of mycotoxins in a multi-mycotoxin LC-MS/MS suite. This involves extraction by shaking in 20:79:1 water : acetonitrile : acetic acid followed by centrifugation, dilution in an equal volume of 79:20:1 water : acetonitrile : acetic acid and filtration of the supernatant. This method was also used or adapted for some of the analytes in this study.

Using the multi-mycotoxin method, mycotoxins extracts are analysed using an LC-MS/MS method which is split into two parts, one with neutral pH mobile phase, which is used for the majority of the analytes in the suite, and one with acidic pH mobile phase which is required to improve the peak shape or response of selected analytes. For some analytes these two multi-mycotoxin methods were adapted in order to maximise response and/or throughput. For some analytes it was necessary to develop a new LC-MS/MS method from scratch. The method used has been described fully in the previous section. Modifications and the reason they were made are given in the results section below for each analyte.

Fully tabulated results are given in Appendix 1. Tables of individual results are given below. Aflatoxins, ochratoxin A, zearalenone, fumonisins, trichothecenes and patulin
methods are already UKAS accredited, although not for all the matrices included in this study. Methods performed well and met QC criteria for plate count, linear regression, and in most cases recovery. Due to the variability in sample types extra spiked samples were included, and in many cases an overspike was carried out for each category sample.

LC-MS/MS analysis is also accredited to UKAS as the accredited protocol for data review, ion ratio for confirmation, and retention time requirements were met in the majority of cases, and where they were not this is indicated in the results tables. In each case the data has been analysed manually and assessed to allow as low an LOD as possible to be calculated for these samples. This is not done routinely in the accredited method to make the method more time efficient but it was thought to be necessary in this case to achieve as low an LOQ as possible. In many cases the range of the calibration series has been changed so the lowest calibration standard is lower than used for normal practice to allow lower LOQs to be reported. The QC data is summarised in Table 15 and Table 16, this gives the range, mean and median of recovery, the LOD where it was possible to calculate it, and LOQ. For those analytes where only LOQ was reported the reason for this is given.
### Table 15. Summarised Recovery and LOD/LOQ data.

<table>
<thead>
<tr>
<th>Analyte</th>
<th>3-Acetyl-Deoxynivalenol</th>
<th>15-Acetyl-Deoxynivalenol</th>
<th>Deoxynivalenol</th>
<th>Diacetoxyscirpenol</th>
<th>Fusarenon_X</th>
<th>HT2_Toxin</th>
<th>Neosolaniol</th>
<th>Nivalenol</th>
<th>T2_Toxin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recovery Range</td>
<td>14-82 %</td>
<td>9-92 %</td>
<td>3-106 %</td>
<td>22-122 %</td>
<td>12-91 %</td>
<td>19-100 %</td>
<td>21-109 %</td>
<td>15-59 %</td>
<td>13-140 %</td>
</tr>
<tr>
<td>Mean Recovery</td>
<td>55%</td>
<td>44%</td>
<td>41%</td>
<td>84%</td>
<td>51%</td>
<td>75%</td>
<td>80%</td>
<td>34%</td>
<td>90%</td>
</tr>
<tr>
<td>Median Recovery</td>
<td>57%</td>
<td>42%</td>
<td>37%</td>
<td>86%</td>
<td>52%</td>
<td>76%</td>
<td>83%</td>
<td>34%</td>
<td>99%</td>
</tr>
<tr>
<td>LOD Range / µg/kg</td>
<td>1.10-7.62</td>
<td>5.45-55.94</td>
<td>1.39-33.25</td>
<td>0.08-0.46</td>
<td>0.70-2.29</td>
<td>1.00-5.39</td>
<td>0.46-2.34</td>
<td>8.51-45.26</td>
<td>0.10-0.78</td>
</tr>
<tr>
<td>LOQ Range / µg/kg</td>
<td>6.08-36.70</td>
<td>5.45-55.94</td>
<td>4.73-166.67</td>
<td>4.11-22.83</td>
<td>5.52-40.35</td>
<td>4.98-26.94</td>
<td>4.57-23.40</td>
<td>8.51-33.54</td>
<td>3.58-38.94</td>
</tr>
</tbody>
</table>

### Table 16. Summarised Recovery and LOQ data.

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Ergocornine</th>
<th>Ergocorninine</th>
<th>Ergocristine</th>
<th>Ergocristinine</th>
<th>Ergocryptine</th>
<th>Ergocryptinine</th>
<th>Ergometrine</th>
<th>Ergometrolnine</th>
<th>Ergosine</th>
<th>Ergosine</th>
<th>Ergotamine</th>
<th>Ergotaminine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recovery Range</td>
<td>78-103 %</td>
<td>70-111 %</td>
<td>77-116 %</td>
<td>78-129 %</td>
<td>77-104 %</td>
<td>78-117 %</td>
<td>80-114 %</td>
<td>82-116 %</td>
<td>82-118 %</td>
<td>76-106 %</td>
<td>80-112 %</td>
<td>77-120 %</td>
</tr>
<tr>
<td>Mean Recovery</td>
<td>97%</td>
<td>101%</td>
<td>108%</td>
<td>115%</td>
<td>98%</td>
<td>108%</td>
<td>97%</td>
<td>93%</td>
<td>93%</td>
<td>98%</td>
<td>102%</td>
<td>110%</td>
</tr>
<tr>
<td>Median Recovery</td>
<td>100%</td>
<td>111%</td>
<td>112%</td>
<td>113%</td>
<td>99%</td>
<td>111%</td>
<td>93%</td>
<td>95%</td>
<td>92%</td>
<td>99%</td>
<td>106%</td>
<td>111%</td>
</tr>
<tr>
<td>LOD Range / µg/kg</td>
<td>0.25-1.14</td>
<td>0.25-0.90</td>
<td>0.25-1.02</td>
<td>0.25-0.88</td>
<td>0.25-1.05</td>
<td>0.25-0.90</td>
<td>0.91-1.26</td>
<td>0.55-1.0</td>
<td>0.25-0.85</td>
<td>0.25-0.85</td>
<td>0.25-0.89</td>
<td>0.25-1.00</td>
</tr>
<tr>
<td>Why LOQ?</td>
<td>Background response</td>
<td>Background response</td>
<td>Background response</td>
<td>Background response</td>
<td>Background response</td>
<td>Background response</td>
<td>Background response</td>
<td>Background response</td>
<td>Background response</td>
<td>Background response</td>
<td>Background response</td>
<td>Background response</td>
</tr>
</tbody>
</table>

### Why LOQ?

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Aflatoxin B1</th>
<th>Aflatoxin B2</th>
<th>Aflatoxin G1</th>
<th>Aflatoxin G2</th>
<th>Ochratoxin A</th>
<th>Aflatoxin M1</th>
<th>Citrinin</th>
<th>Moniliformin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recovery Range</td>
<td>9-116 %</td>
<td>12-158 %</td>
<td>8-103 %</td>
<td>10-116 %</td>
<td>32-100 %</td>
<td>43-85 %</td>
<td>46-93 %</td>
<td>3-76 %</td>
</tr>
<tr>
<td>Mean Recovery</td>
<td>78%</td>
<td>93%</td>
<td>70%</td>
<td>67%</td>
<td>78%</td>
<td>57%</td>
<td>78%</td>
<td>20%</td>
</tr>
<tr>
<td>Median Recovery</td>
<td>82%</td>
<td>96%</td>
<td>81%</td>
<td>80%</td>
<td>82%</td>
<td>60%</td>
<td>79%</td>
<td>8%</td>
</tr>
<tr>
<td>LOD Range / µg/kg</td>
<td>0.17-2.26</td>
<td>0.14-1.60</td>
<td>0.17-2.44</td>
<td>0.17-1.97</td>
<td>0.20-0.56</td>
<td>0.02-0.05</td>
<td>1.07-2.16</td>
<td>1.31-39.50</td>
</tr>
<tr>
<td>Why LOQ?</td>
<td>Usual method of reporting</td>
<td>Usual method of reporting</td>
<td>Usual method of reporting</td>
<td>Usual method of reporting</td>
<td>Usual method of reporting</td>
<td>Usual method of reporting</td>
<td>Peaks in several samples</td>
<td>Peaks in most samples</td>
</tr>
</tbody>
</table>
8.2. Validation of New Methods

The sterigmatocystin method was validated as part of another project (FSA/EFSA sterigmatocystin survey).

The methods for citrinin, cyclopiazonic acid and moniliformin were each validated by the analysis of replicate spiked samples (n = 6) once the optimum sample preparation and analysis conditions had been developed. Results for the validation of these methods are given in the results section for each of the analytes in the sections below.

8.3. Aflatoxins

Aflatoxins were analysed using the established accredited method, although the method has not been fully validated for all the matrices tested in this study. The results for aflatoxins are summarised in Table 17 and Table 18. Aflatoxin B1, B2, G1 and G2 were not detected in any sample. Limits of quantification calculated ranged from 0.13 µg/kg for aflatoxin B2 in dried fruit to 2.41 µg/kg for aflatoxin G1 in sugar confectionary. The majority of the calculated limits of quantification were at or around 0.2 to 0.3 µg/kg. The higher limits of quantification were a result of lower recovery values.

All dairy and egg products were analysed for aflatoxin M1 only, as this is the metabolite of aflatoxin B1 that would be present in these products if the animals had been exposed to significant aflatoxin concentrations in their diets. None of the samples analysed for aflatoxin M1 contained residues above the calculated LOQs that ranged from 0.02 to 0.05 µg/kg. Offal samples were analysed for aflatoxins B1, B2, G1 and G2 as these are the analytes these products are routinely test for in the UK for the Statutory Veterinary Residues Monitoring programme under EC Directive 96/23 - Measures to monitor certain substances and residues thereof in live animals and animal products.

8.4. Ochratoxin A

Ochratoxin A was analysed using the established accredited method, although the method has not been fully validated for all the matrices tested in this study. The results for ochratoxin A are summarised in Table 17 and Table 18. Five samples contained residues of ochratoxin A. The highest level found was 5.6 µg/kg in fruit & vegetable juices, although this result was corrected for a low recovery (32 %). The second highest value was 1.65 µg/kg in the dried fruit samples (100 % recovery). The other samples that contained residues were herbs and spices (0.63 µg/kg), brown bread (0.53 µg/kg) and granary bread (0.45 µg/kg). All levels measured were below the maximum permitted levels according to Regulation (EC) 1881/2006 (and amendments).
## Table 17. Aflatoxins and Ochratoxin A Results – Part 1.

<table>
<thead>
<tr>
<th>Group</th>
<th>Category</th>
<th>LIMS Number</th>
<th>Aflatoxin B1</th>
<th>Aflatoxin B2</th>
<th>Aflatoxin G1</th>
<th>Aflatoxin G2</th>
<th>Ochratoxin A</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Concentration (µg/kg)</td>
<td>Concentration (µg/kg)</td>
<td>Concentration (µg/kg)</td>
<td>Concentration (µg/kg)</td>
<td>Concentration (µg/kg)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>&lt; LOQ (&lt; 0.24)</td>
<td>&lt; LOQ (&lt; 0.19)</td>
<td>&lt; LOQ (&lt; 0.25)</td>
<td>&lt; LOQ (&lt; 0.22)</td>
<td>&lt; LOQ (&lt; 0.23)</td>
</tr>
<tr>
<td>Bread</td>
<td>White sliced bread</td>
<td>S14-042656</td>
<td>Result Recovery</td>
<td>Recovery</td>
<td>Recovery</td>
<td>Recovery</td>
<td>Recovery</td>
</tr>
<tr>
<td></td>
<td>White sliced bread</td>
<td>S14-042657</td>
<td>Result Recovery</td>
<td>Recovery</td>
<td>Recovery</td>
<td>Recovery</td>
<td>Recovery</td>
</tr>
<tr>
<td></td>
<td>Brown bread</td>
<td>S14-042658</td>
<td>Result Recovery</td>
<td>Recovery</td>
<td>Recovery</td>
<td>Recovery</td>
<td>Recovery</td>
</tr>
<tr>
<td></td>
<td>Wholemeal and granary bread</td>
<td>S14-042659</td>
<td>Result Recovery</td>
<td>Recovery</td>
<td>Recovery</td>
<td>Recovery</td>
<td>Recovery</td>
</tr>
<tr>
<td></td>
<td>Other bread</td>
<td>S14-042660</td>
<td>Result Recovery</td>
<td>Recovery</td>
<td>Recovery</td>
<td>Recovery</td>
<td>Recovery</td>
</tr>
<tr>
<td></td>
<td>Offals</td>
<td></td>
<td>Result Recovery</td>
<td>Recovery</td>
<td>Recovery</td>
<td>Recovery</td>
<td>Recovery</td>
</tr>
<tr>
<td></td>
<td>Lamb's liver</td>
<td>S14-042674</td>
<td>Result Recovery</td>
<td>Recovery</td>
<td>Recovery</td>
<td>Recovery</td>
<td>Recovery</td>
</tr>
<tr>
<td></td>
<td>Pigs liver</td>
<td>S14-042675</td>
<td>Result Recovery</td>
<td>Recovery</td>
<td>Recovery</td>
<td>Recovery</td>
<td>Recovery</td>
</tr>
<tr>
<td></td>
<td>Other liver</td>
<td>S14-042676</td>
<td>Result Recovery</td>
<td>Recovery</td>
<td>Recovery</td>
<td>Recovery</td>
<td>Recovery</td>
</tr>
<tr>
<td></td>
<td>Kidney</td>
<td>S14-042677</td>
<td>Result Recovery</td>
<td>Recovery</td>
<td>Recovery</td>
<td>Recovery</td>
<td>Recovery</td>
</tr>
<tr>
<td></td>
<td>Other offals (excluding kidney and liver)</td>
<td>S14-042678</td>
<td>Result Recovery</td>
<td>Recovery</td>
<td>Recovery</td>
<td>Recovery</td>
<td>Recovery</td>
</tr>
<tr>
<td></td>
<td>Group sample</td>
<td>S14-042679</td>
<td>Result Recovery</td>
<td>Recovery</td>
<td>Recovery</td>
<td>Recovery</td>
<td>Recovery</td>
</tr>
<tr>
<td></td>
<td>Oils and fats</td>
<td></td>
<td>Result Recovery</td>
<td>Recovery</td>
<td>Recovery</td>
<td>Recovery</td>
<td>Recovery</td>
</tr>
<tr>
<td></td>
<td>Vegetable oils</td>
<td>S14-042680</td>
<td>Result Recovery</td>
<td>Recovery</td>
<td>Recovery</td>
<td>Recovery</td>
<td>Recovery</td>
</tr>
<tr>
<td></td>
<td>Eggs</td>
<td>S14-042681</td>
<td>Result Recovery</td>
<td>Recovery</td>
<td>Recovery</td>
<td>Recovery</td>
<td>Recovery</td>
</tr>
<tr>
<td></td>
<td>Sugar and preserves</td>
<td></td>
<td>Result Recovery</td>
<td>Recovery</td>
<td>Recovery</td>
<td>Recovery</td>
<td>Recovery</td>
</tr>
<tr>
<td></td>
<td>Chocolates confectionery</td>
<td>S14-042682</td>
<td>Result Recovery</td>
<td>Recovery</td>
<td>Recovery</td>
<td>Recovery</td>
<td>Recovery</td>
</tr>
<tr>
<td></td>
<td>Sugar confectionery</td>
<td>S14-042683</td>
<td>Result Recovery</td>
<td>Recovery</td>
<td>Recovery</td>
<td>Recovery</td>
<td>Recovery</td>
</tr>
<tr>
<td></td>
<td>Other vegetables</td>
<td></td>
<td>Result Recovery</td>
<td>Recovery</td>
<td>Recovery</td>
<td>Recovery</td>
<td>Recovery</td>
</tr>
<tr>
<td></td>
<td>Dried pulses</td>
<td>S14-042684</td>
<td>Result Recovery</td>
<td>Recovery</td>
<td>Recovery</td>
<td>Recovery</td>
<td>Recovery</td>
</tr>
<tr>
<td></td>
<td>Herbs, spices</td>
<td>S14-042685</td>
<td>Result Recovery</td>
<td>Recovery</td>
<td>Recovery</td>
<td>Recovery</td>
<td>Recovery</td>
</tr>
<tr>
<td></td>
<td>Fruit products</td>
<td></td>
<td>Result Recovery</td>
<td>Recovery</td>
<td>Recovery</td>
<td>Recovery</td>
<td>Recovery</td>
</tr>
<tr>
<td></td>
<td>Dried fruits</td>
<td>S14-042686</td>
<td>Result Recovery</td>
<td>Recovery</td>
<td>Recovery</td>
<td>Recovery</td>
<td>Recovery</td>
</tr>
<tr>
<td></td>
<td>Fruit juices and vegetable juices</td>
<td>S14-042687</td>
<td>Result Recovery</td>
<td>Recovery</td>
<td>Recovery</td>
<td>Recovery</td>
<td>Recovery</td>
</tr>
</tbody>
</table>
### Table 18. Aflatoxins and Ochratoxin A Results – Part 2.

<table>
<thead>
<tr>
<th>Group</th>
<th>Category</th>
<th>LIMS Number</th>
<th>Ochratoxin / Aflatoxin Concentration (µg/kg)</th>
<th>Aflatoxin B1 Concentration (µg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Concentrations are corrected for recovery.</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>QC sample recovery value.</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Concentrations are corrected for recovery.</td>
<td></td>
</tr>
</tbody>
</table>

#### Aflatoxin M1 Concentrations

- **LI M S**: µ g lk g

#### Ochratoxin

- **Aflatoxin 81**: Aflatoxin 82: Aflatoxin G1: Aflatoxin G2: Ochratoxin A

#### Recovery Results

- **Result**: < LOQ (< 0.46) < LOQ (< 0.40) < LOQ (< 0.35) < LOQ (< 1.30) < LOQ (< 0.24)

#### Non-alcohol - Beverages (with bottled water)

<p>| | | | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
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<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>102</td>
<td>Tea</td>
<td>S14-042954</td>
<td>Result</td>
<td>Recovery</td>
<td>&lt; LOQ (&lt; 0.46)</td>
</tr>
<tr>
<td>103</td>
<td>Tea</td>
<td>S14-042955</td>
<td>Result</td>
<td>Recovery</td>
<td>&lt; LOQ (&lt; 0.46)</td>
</tr>
<tr>
<td>104</td>
<td>Instant coffee</td>
<td>S14-042956</td>
<td>Result</td>
<td>Recovery</td>
<td>&lt; LOQ (&lt; 0.46)</td>
</tr>
<tr>
<td>105</td>
<td>Ground coffee</td>
<td>S14-042957</td>
<td>Result</td>
<td>Recovery</td>
<td>&lt; LOQ (&lt; 0.61)</td>
</tr>
<tr>
<td>106</td>
<td>Takeaway coffee</td>
<td>S14-042958</td>
<td>Result</td>
<td>Recovery</td>
<td>&lt; LOQ (&lt; 0.61)</td>
</tr>
<tr>
<td>107</td>
<td>Brandied food drink</td>
<td>S14-042959</td>
<td>Result</td>
<td>Recovery</td>
<td>&lt; LOQ (&lt; 0.61)</td>
</tr>
<tr>
<td>108</td>
<td>Cocoa, drinking chocolate</td>
<td>S14-042960</td>
<td>Result</td>
<td>Recovery</td>
<td>&lt; LOQ (&lt; 0.75)</td>
</tr>
<tr>
<td>109</td>
<td>Almonds in milk</td>
<td>S14-042963</td>
<td>Result</td>
<td>Recovery</td>
<td>&lt; LOQ (&lt; 0.31)</td>
</tr>
</tbody>
</table>

#### Milk

<p>| | | | | | |</p>
<table>
<thead>
<tr>
<th></th>
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<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>110</td>
<td>Whole (full milk)</td>
<td>S14-042964</td>
<td>Result</td>
<td>Recovery</td>
<td>&lt; LOQ (&lt; 0.02)</td>
</tr>
<tr>
<td>111</td>
<td>Skimmed/Semi skimmed milk (milk)</td>
<td>S14-042965</td>
<td>Result</td>
<td>Recovery</td>
<td>&lt; LOQ (&lt; 0.02)</td>
</tr>
<tr>
<td>112</td>
<td>Group</td>
<td>NA</td>
<td>S14-042845</td>
<td>Group sample</td>
<td>Result</td>
</tr>
</tbody>
</table>

#### Dairy products

<p>| | | | | | |</p>
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<thead>
<tr>
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</tr>
</thead>
<tbody>
<tr>
<td>114</td>
<td>Condensed milk or Evaporated</td>
<td>S14-042965</td>
<td>Result</td>
<td>Recovery</td>
<td>&lt; LOQ (&lt; 0.02)</td>
</tr>
<tr>
<td>115</td>
<td>Instant milk</td>
<td>S14-042966</td>
<td>Result</td>
<td>Recovery</td>
<td>&lt; LOQ (&lt; 0.02)</td>
</tr>
<tr>
<td>116</td>
<td>Natural cheese</td>
<td>S14-042965</td>
<td>Result</td>
<td>Recovery</td>
<td>&lt; LOQ (&lt; 0.02)</td>
</tr>
<tr>
<td>117</td>
<td>Processed cheese</td>
<td>S14-042966</td>
<td>Result</td>
<td>Recovery</td>
<td>&lt; LOQ (&lt; 0.02)</td>
</tr>
<tr>
<td>118</td>
<td>Butti</td>
<td>S14-042967</td>
<td>Result</td>
<td>Recovery</td>
<td>&lt; LOQ (&lt; 0.02)</td>
</tr>
<tr>
<td>119</td>
<td>Ice-cream</td>
<td>S14-042968</td>
<td>Result</td>
<td>Recovery</td>
<td>&lt; LOQ (&lt; 0.02)</td>
</tr>
<tr>
<td>120</td>
<td>Yogurt</td>
<td>S14-042969</td>
<td>Result</td>
<td>Recovery</td>
<td>&lt; LOQ (&lt; 0.02)</td>
</tr>
<tr>
<td>121</td>
<td>Cream</td>
<td>S14-042970</td>
<td>Result</td>
<td>Recovery</td>
<td>&lt; LOQ (&lt; 0.02)</td>
</tr>
<tr>
<td>122</td>
<td>Canned milk products</td>
<td>S14-042971</td>
<td>Result</td>
<td>Recovery</td>
<td>&lt; LOQ (&lt; 0.02)</td>
</tr>
<tr>
<td>123</td>
<td>Group</td>
<td>NA</td>
<td>S14-042846</td>
<td>Group sample</td>
<td>Result</td>
</tr>
</tbody>
</table>

#### Nuts

<p>| | | | | | |</p>
<table>
<thead>
<tr>
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</tr>
</thead>
<tbody>
<tr>
<td>124</td>
<td>Ground nuts including peanut butter</td>
<td>S14-042972</td>
<td>Result</td>
<td>Recovery</td>
<td>&lt; LOQ (&lt; 0.26)</td>
</tr>
<tr>
<td>125</td>
<td>Tree nuts</td>
<td>S14-042973</td>
<td>Result</td>
<td>Recovery</td>
<td>&lt; LOQ (&lt; 0.26)</td>
</tr>
<tr>
<td>126</td>
<td>Almonds</td>
<td>S14-042974</td>
<td>Result</td>
<td>Recovery</td>
<td>&lt; LOQ (&lt; 0.26)</td>
</tr>
</tbody>
</table>

#### Alcoholic drinks

<p>| | | | | | |</p>
<table>
<thead>
<tr>
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<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>127</td>
<td>Beer</td>
<td>S14-042975</td>
<td>Result</td>
<td>Recovery</td>
<td>&lt; LOQ (&lt; 0.26)</td>
</tr>
<tr>
<td>128</td>
<td>Wines</td>
<td>S14-042976</td>
<td>Result</td>
<td>Recovery</td>
<td>&lt; LOQ (&lt; 0.26)</td>
</tr>
<tr>
<td>129</td>
<td>Cider</td>
<td>S14-042977</td>
<td>Result</td>
<td>Recovery</td>
<td>&lt; LOQ (&lt; 0.26)</td>
</tr>
<tr>
<td>130</td>
<td>Alcopops and cocktails</td>
<td>S14-042978</td>
<td>Result</td>
<td>Recovery</td>
<td>&lt; LOQ (&lt; 0.26)</td>
</tr>
<tr>
<td>131</td>
<td>Other drinks (not potato based)</td>
<td>S14-042979</td>
<td>Result</td>
<td>Recovery</td>
<td>&lt; LOQ (&lt; 0.26)</td>
</tr>
</tbody>
</table>

#### Snacks

<p>| | | | | | |</p>
<table>
<thead>
<tr>
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</thead>
<tbody>
<tr>
<td>132</td>
<td>Sandwiches</td>
<td>S14-042980</td>
<td>Result</td>
<td>Recovery</td>
<td>&lt; LOQ (&lt; 0.26)</td>
</tr>
<tr>
<td>133</td>
<td>Bread</td>
<td>S14-042981</td>
<td>Result</td>
<td>Recovery</td>
<td>&lt; LOQ (&lt; 0.26)</td>
</tr>
<tr>
<td>134</td>
<td>Group</td>
<td>NA</td>
<td>S14-042852</td>
<td>Group sample</td>
<td>Result</td>
</tr>
</tbody>
</table>

Grey shading indicates that no analysis was requested.
8.5. **Zearalenone**

Zearalenone was analysed using the established accredited method, although the method has not been fully validated for all the matrices tested in this study. Full zearalenone results are given in Table 19. Five samples contained residues above the LOD but below the LOQ, levels ranged from 0.57 to 1.92 µg/kg, although these values are not quantitative. The pizza sample contained a level of 16.5 µg/kg corrected for a recovery for 47 %, this was the highest level measured in all the samples. All levels measured were well below the maximum permitted levels in legislation.

8.6. **Fumonisins B₁, B₂ and B₃**

The fumonisins were analysed using the established accredited acidic multi-mycotoxin method. Full results are given in Table 20.

Fumonisin B₁ was detected in the sample of herbs and spices at a level of 5.53 µg/kg, below the LOQ of 7.15 µg/kg. Fumonisins were not detected in any other samples above the limit of detection (LOD). Limits of detection for Fumonisin B₁ ranged from 3.87 µg/kg for beers and cider to 7.95 µg/kg for flour, and chocolate biscuits had a much higher LOD of 37.1 µg/kg. For Fumonisin B₂ LODs were in the range 3.4 to 8.9 µg/kg apart from chocolate biscuits where it was 36.8 µg/kg. For fumonisin B₃ LODs were in the range 3.1 to 6.5 µg/kg, with a LOD in chocolate biscuits of 31.5 µg/kg.
Table 19. Zearalenone Results.

<table>
<thead>
<tr>
<th>Group</th>
<th>Category</th>
<th>LIMS Number</th>
<th>Zearalenone Concentration / µg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Bread</td>
<td>S14-042856</td>
<td>Result: &lt; LOD (&lt; 0.34), Recovery: 87% ^</td>
</tr>
<tr>
<td>2</td>
<td>White sliced bread</td>
<td>S14-042857</td>
<td>Result: &lt; LOD (&lt; 0.42), Recovery: 72% '</td>
</tr>
<tr>
<td>3</td>
<td>Brown bread</td>
<td>S14-042858</td>
<td>Result: &lt; LOD (&lt; 0.42), Recovery: 72%</td>
</tr>
<tr>
<td>4</td>
<td>Wholemeal and granary bread</td>
<td>S14-042859</td>
<td>Result: &lt; LOD (&lt; 0.34), Recovery: 87%</td>
</tr>
<tr>
<td>5</td>
<td>Other bread</td>
<td>S14-042860</td>
<td>Result: &lt; LOD (&lt; 0.42), Recovery: 72% '</td>
</tr>
<tr>
<td>NA</td>
<td>Group sample</td>
<td>S14-042828</td>
<td>Result: &lt; LOD (&lt; 0.71), Recovery: 42%</td>
</tr>
<tr>
<td>6</td>
<td>Miscellaneous cereals</td>
<td>S14-042861</td>
<td>Result: &lt; LOD (&lt; 0.34), Recovery: 87% ^</td>
</tr>
<tr>
<td>7</td>
<td>Flour</td>
<td>S14-042862</td>
<td>Result: &lt; LOD (&lt; 0.29), Recovery: 102% **</td>
</tr>
<tr>
<td>8</td>
<td>Buns, cakes and pastries</td>
<td>S14-042863</td>
<td>Result: &lt; LOD (&lt; 0.29), Recovery: 102% **</td>
</tr>
<tr>
<td>9</td>
<td>Savoury biscuits</td>
<td>S14-042864</td>
<td>Result: 0.57 &lt; LOQ (&lt; 2.46), Recovery: 102%</td>
</tr>
<tr>
<td>10</td>
<td>Sweet biscuits</td>
<td>S14-042865</td>
<td>Result: 0.85 &lt; LOQ (&lt; 2.46), Recovery: 102% **</td>
</tr>
<tr>
<td>11</td>
<td>Chocolate biscuits</td>
<td>S14-042866</td>
<td>Result: &lt; LOD (&lt; 0.34), Recovery: 87% ^</td>
</tr>
<tr>
<td>12</td>
<td>Breakfast cereals</td>
<td>S14-042867</td>
<td>Result: &lt; LOD (&lt; 0.41), Recovery: 73%</td>
</tr>
<tr>
<td>13</td>
<td>Rice</td>
<td>S14-042868</td>
<td>Result: &lt; LOD (&lt; 0.29), Recovery: 102% **</td>
</tr>
<tr>
<td>14</td>
<td>Other cereal products</td>
<td>S14-042869</td>
<td>Result: &lt; LOD (&lt; 0.41), Recovery: 73% ***</td>
</tr>
<tr>
<td>15</td>
<td>Pasta</td>
<td>S14-042870</td>
<td>Result: 16.45, Recovery: 47%</td>
</tr>
<tr>
<td>NA</td>
<td>Group sample</td>
<td>S14-042829</td>
<td>Result: &lt; LOD (&lt; 0.70), Recovery: 43%</td>
</tr>
<tr>
<td>16</td>
<td>Potatoes</td>
<td>S14-042924</td>
<td>Result: &lt; LOD (&lt; 0.78), Recovery: 38%</td>
</tr>
<tr>
<td>17</td>
<td>Fresh potatoes</td>
<td>S14-042925</td>
<td>Result: 1.92 &lt; LOQ (&lt; 6.54), Recovery: 38% ^</td>
</tr>
<tr>
<td>NA</td>
<td>Group sample</td>
<td>S14-042839</td>
<td>Result: &lt; LOD (&lt; 0.66), Recovery: 45%</td>
</tr>
<tr>
<td>18</td>
<td>Potato products</td>
<td>S14-042933</td>
<td>Result: 1.33 &lt; LOQ (&lt; 6.95), Recovery: 36%</td>
</tr>
<tr>
<td>19</td>
<td>Herbs, spices</td>
<td>S14-042934</td>
<td>Result: &lt; LOD (&lt; 0.83), Recovery: 30% **</td>
</tr>
<tr>
<td>20</td>
<td>Dried pulses</td>
<td>S14-042959</td>
<td>Result: &lt; LOD (&lt; 0.60), Recovery: 50%</td>
</tr>
<tr>
<td>21</td>
<td>Branded food drinks</td>
<td>S14-042960</td>
<td>Result: &lt; LOD (&lt; 1.15), Recovery: 26%</td>
</tr>
<tr>
<td>22</td>
<td>Cocoa, drinking chocolate</td>
<td>S14-042963</td>
<td>Result: &lt; LOD (&lt; 0.53), Recovery: 57%</td>
</tr>
<tr>
<td>23</td>
<td>Alternatives to milk</td>
<td>S14-042978</td>
<td>Result: &lt; LOD (&lt; 0.43), Recovery: 70%</td>
</tr>
<tr>
<td>24</td>
<td>Beer</td>
<td>S14-042979</td>
<td>Result: &lt; LOD (&lt; 0.43), Recovery: 70% **</td>
</tr>
<tr>
<td>25</td>
<td>Other snacks (not potato based)</td>
<td>S14-042985</td>
<td>Result: 1.09 &lt; LOQ (&lt; 6.73), Recovery: 37%</td>
</tr>
<tr>
<td>NA</td>
<td>Group sample</td>
<td>S14-042852</td>
<td>Result: &lt; LOD (&lt; 0.81), Recovery: 37% **</td>
</tr>
<tr>
<td>Group</td>
<td>Category</td>
<td>LIMS Number</td>
<td>Fumonisin Concentration / µg/kg</td>
</tr>
<tr>
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<td>----------</td>
<td>---------------</td>
<td>---------------------------------</td>
</tr>
<tr>
<td></td>
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<td>Fumonisin B1</td>
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<tr>
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<td>Result</td>
<td>Recovery</td>
</tr>
<tr>
<td>1</td>
<td>Bread</td>
<td>S14-042856</td>
<td>&lt; LOD (&lt; 6.69)</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>S14-042857</td>
<td>&lt; LOD (&lt; 6.69)</td>
</tr>
<tr>
<td>3</td>
<td>Brown bread</td>
<td>S14-042858</td>
<td>&lt; LOD (&lt; 6.69)</td>
</tr>
<tr>
<td>4</td>
<td>Wholemeal and granary bread</td>
<td>S14-042859</td>
<td>&lt; LOD (&lt; 6.69)</td>
</tr>
<tr>
<td>5</td>
<td>Other bread</td>
<td>S14-042860</td>
<td>&lt; LOD (&lt; 6.69)</td>
</tr>
<tr>
<td>NA</td>
<td>Group sample</td>
<td>S14-042828</td>
<td>&lt; LOD (&lt; 6.69)</td>
</tr>
<tr>
<td>6</td>
<td>Flour</td>
<td>S14-042861</td>
<td>&lt; LOD (&lt; 7.96)</td>
</tr>
<tr>
<td>7</td>
<td>Buns, cakes and pastries</td>
<td>S14-042862</td>
<td>&lt; LOD (&lt; 6.57)</td>
</tr>
<tr>
<td>8</td>
<td>Savoury biscuits</td>
<td>S14-042863</td>
<td>&lt; LOD (&lt; 6.57)</td>
</tr>
<tr>
<td>9</td>
<td>Sweet biscuits</td>
<td>S14-042864</td>
<td>&lt; LOD (&lt; 6.57)</td>
</tr>
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<td>Chocolate biscuits</td>
<td>S14-042865</td>
<td>&lt; LOD (&lt; 37.05)</td>
</tr>
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<td>11</td>
<td>Breakfast cereals</td>
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</tr>
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<td>12</td>
<td>Rice</td>
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</tr>
<tr>
<td>13</td>
<td>Other cereal products</td>
<td>S14-042868</td>
<td>&lt; LOD (&lt; 6.57)</td>
</tr>
<tr>
<td>14</td>
<td>Pasta</td>
<td>S14-042869</td>
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</tr>
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<td>15</td>
<td>Pizza</td>
<td>S14-042870</td>
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<td>S14-042829</td>
<td>&lt; LOD (&lt; 3.94)</td>
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<td>16</td>
<td>Fresh potatoes</td>
<td>S14-042924</td>
<td>&lt; LOD (&lt; 4.02)</td>
</tr>
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<td>17</td>
<td>Potato products</td>
<td>S14-042925</td>
<td>&lt; LOD (&lt; 4.02)</td>
</tr>
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<td>&lt; LOD (&lt; 4.02)</td>
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<td>18</td>
<td>Dried pulses</td>
<td>S14-042933</td>
<td>&lt; LOD (&lt; 4.26)</td>
</tr>
<tr>
<td>19</td>
<td>Herbs, spices</td>
<td>S14-042934</td>
<td>5.53 &lt; LOD (&lt; 7.15)</td>
</tr>
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<td>20</td>
<td>Branded food drinks</td>
<td>S14-042959</td>
<td>&lt; LOD (&lt; 4.10)</td>
</tr>
<tr>
<td>21</td>
<td>Alcoholic drinks</td>
<td>S14-042963</td>
<td>&lt; LOD (&lt; 4.27)</td>
</tr>
<tr>
<td>22</td>
<td>Beer</td>
<td>S14-042978</td>
<td>&lt; LOD (&lt; 3.87)</td>
</tr>
<tr>
<td>23</td>
<td>Cider</td>
<td>S14-042979</td>
<td>&lt; LOD (&lt; 3.87)</td>
</tr>
<tr>
<td>24</td>
<td>Wine</td>
<td>S14-042980</td>
<td>&lt; LOD (&lt; 3.87)</td>
</tr>
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<td>25</td>
<td>Snacks</td>
<td>S14-042985</td>
<td>&lt; LOD (&lt; 6.84)</td>
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<td>26</td>
<td>Sandwiches</td>
<td>S14-042988</td>
<td>&lt; LOD (&lt; 4.09)</td>
</tr>
<tr>
<td>NA</td>
<td>Group sample</td>
<td>S14-042852</td>
<td>&lt; LOD (&lt; 4.09)</td>
</tr>
</tbody>
</table>

Concentrations are corrected for recovery. * Group sample recovery value. ** Wine sample recovery value.
8.7. Trichothecenes

Samples were analysed by UPLC-MS/MS using a method that allowed the separation of nine trichothecenes - deoxynivalenol, 3-acetyldeoxynivalenol, 15-acetyldeoxynivalenol, nivalenol, fusarenon-X, diacetoxyscirpenol, neosolaniol, T-2 toxin, HT-2 toxin. The existing neutral mobile phase multi-mycotoxin LC-MS method did not give separation of the isomers 3- and 15-acetyldeoxynivalenol and this could not be achieved by changing the gradient profile so a new column type, the Restek Raptor Biphenyl 2.7 µm (100 x 2.1 mm), was selected to replace the Waters Acquity HSS T3 1.8 µm (100 x 2.1 mm). The mobile phases were not changed but the gradient was re-optimised to give separation of these two isomers. The injection volume was increased because the aqueous content of the sample extracts was higher than in the usual multi-mycotoxin method so it was possible to inject more without deterioration of the peak shapes of early-eluting compounds.

Full trichothecene results are given in Table 21. Nine trichothecenes were included in the analysis. 3-Acetyl-deoxynivalenol, 15-acetyl-deoxynivalenol, and nivalenol were not detected in any sample above the LOD. In addition neosolaniol was not detected in any sample requested above the LOD, however a few additional analyses (not originally requested by the FSA) have been included and neosolaniol was found in the vegetable oils sample between LOD and LOQ at level of 0.73 µg/kg.

T-2 and HT-2 toxin were also detected in a number of samples above LOD but below LOQ. Most samples with detectable residues were cereal products, however they were both detected in vegetable oils and cider, and T-2 toxin was seen in the potatoes group sample and in dried pulses.

Deoxynivalenol was detected in all cereal products, snack and sandwiches at levels from 11.2 to 166 µg/kg. It was also seen at concentrations between LOD and LOQ in herbs and spices, vegetable oil and beer. The recovery of some of the initial analysis was low, as while there are regulatory limits for DON in cereal products the accepted standardised method has only been validated for flours rather than processed products, e.g. bread. To confirm the initial results the analysis was repeated using \(^{13}\)C-deoxynivalenol internal standard to control the measurement. This second analysis confirmed the presence of deoxynivalenol in all the samples tested, and in most cases the concentration found was in good agreement. The apparent recovery values reported for the second analysis also appear low, but in fact the result reported was inherently corrected by the presence of the internal standard, so these recovery values were not used for correction. It is reassuring that the reported values between the two analyses are in good agreement. The largest variation was for the sample of ‘other bread’ where the initial result was 166 µg/kg but the repeat analysis...
found 79 µg/kg. This is around 50 % difference which is close to the measurement uncertainty for this measurement at 166 µg/kg of 39 % (64 µg/kg). 

The difference could also in some part be due to the sample preparation process as the samples were prepared initially for metals analysis not mycotoxins, and may not have been homogenous for mycotoxins as the sample contained lumps and pieces of crust and seeds were visible. It has been shown that to produce a homogenous sample for mycotoxin analysis samples should be slurried with water or milled to a very small particle size (< 500 µm sieve size) followed by mixing [Spanjer et al, 2006]. This was not done for these samples as they were initially intended for metals analysis, the slurry method would have made some samples incompatible with the analytical methods used, and ultimately this additional preparation would have added a considerable resource burden and cost to the whole project that it was not possible to meet. For the majority of samples the homogenisation was sufficient, but for a small number, such as in this case it may have resulted in less than homogenous test portions.
Table 21. Trichothecene Results

<table>
<thead>
<tr>
<th>Group</th>
<th>Category</th>
<th>LMS Number</th>
<th>3-Acetyl-Deoxynivalenol</th>
<th>15-Acetyl-Deoxynivalenol</th>
<th>Deoxynivalenol</th>
<th>Original Result</th>
<th>Result</th>
<th>Recovery</th>
<th>Recovery</th>
</tr>
</thead>
</table>
| 1 Bread                        | White sliced bread   | S14-042856| < LOD (< 1.50)          | < LOD (< 1.60)           | 37.82          | 60.13          | < LOD (< 1.12)| < LOD (< 0.87)| < LOD (< 1.41)|< LOD (< 2.30)| < LOD (< 17.35)| 0.14 < LOD (< 5.48)
| 1 Bread                        | White unsliced bread | S14-042857| < LOD (< 0.47)          | < LOD (< 0.54)           | 72.63          | 64.15          | < LOD (< 0.99)| < LOD (< 1.51)| < LOD (< 1.04)|< LOD (< 0.58)| < LOD (< 12.33)| 0.19 < LOD (< 0.45)
| 1 Bread                        | Brown bread          | S14-042858| < LOD (< 1.59)          | < LOD (< 1.43)           | 48.05          | 106.3          | < LOD (< 1.10)| < LOD (< 0.96)| < LOD (< 2.06)|< LOD (< 0.59)| < LOD (< 3.27)| 0.12 < LOD (< 4.82)
| 1 Bread                        | Wheaten and granary bread | S14-042859| < LOD (< 1.04)          | < LOD (< 0.88)           | 159.27         | 99.23          | < LOD (< 2.11)| < LOD (< 0.05)| 1.09 < LOD (< 0.05)|< LOD (< 0.84)| < LOD (< 0.44)|
| 1 Bread                        | Other bread          | S14-042860| < LOD (< 1.43)          | < LOD (< 0.92)           | 166.4          | 76.34          | < LOD (< 1.05)| < LOD (< 2.29)| < LOD (< 1.76)|< LOD (< 0.95)| < LOD (< 23.47)| 0.19 < LOD (< 0.72)
| NA Group sample                | Beer                 | S14-042862| < LOD (< 1.38)          | < LOD (< 1.41)           | 77.42          | 76.73          | < LOD (< 1.09)| < LOD (< 1.57)| < LOD (< 1.53)|< LOD (< 0.82)| < LOD (< 13.95)| 0.19 < LOD (< 0.40)
| NA Group sample                | Flour                | S14-042861|                          |                          | 27.24          | 66.44          | < LOD (< 1.05)| < LOD (< 0.95)| 1.21 < LOD (< 1.04)|< LOD (< 0.49)| < LOD (< 5.81)| 0.15 < LOD (< 3.58)
| 2 Miscellaneous cereals       | Buff, cakes and pastries | S14-042862| < LOD (< 1.38)          | < LOD (< 1.74)           | 19.72          | 66.34          | < LOD (< 1.11)| < LOD (< 1.01)| < LOD (< 1.24)|< LOD (< 0.52)| < LOD (< 1.91)|
| 2 Miscellaneous cereals       | Savoury biscuits     | S14-042863| < LOD (< 2.93)          | < LOD (< 2.04)           | 153.2          | 51.63          | < LOD (< 1.32)| < LOD (< 0.85)| < LOD (< 2.37)|< LOD (< 1.18)| < LOD (< 3.34)| 0.19 < LOD (< 0.23)
| 2 Miscellaneous cereals       | Sweet biscuits       | S14-042864| < LOD (< 1.51)          | < LOD (< 0.66)           | 23.75          | 60.52          | < LOD (< 1.01)| < LOD (< 0.92)| 1.5 < LOD (< 0.65)|< LOD (< 0.93)| < LOD (< 0.33)| < LOD (< 0.73)| 0.19 < LOD (< 0.48)
| 2 Miscellaneous cereals       | Breakfast cereals    | S14-042866| < LOD (< 1.70)          | < LOD (< 1.49)           | 34.70          | 50.65          | < LOD (< 1.11)| < LOD (< 0.86)| < LOD (< 0.61)|< LOD (< 0.32)| < LOD (< 18.90)| 0.19 < LOD (< 1.44)
| 2 Miscellaneous cereals       | Rice                 | S14-042867| < LOD (< 1.38)          | < LOD (< 0.48)           | 60.52          | 61.93          | < LOD (< 1.02)| < LOD (< 0.90)| < LOD (< 1.24)|< LOD (< 0.51)| < LOD (< 19.99)| 0.19 < LOD (< 0.20)
| 2 Miscellaneous cereals       | Other cereal products| S14-042868| < LOD (< 1.77)          | < LOD (< 0.94)           | 27.61          | 50.76          | < LOD (< 1.01)| < LOD (< 0.70)| < LOD (< 0.10)|< LOD (< 0.46)| < LOD (< 10.30)| 0.19 < LOD (< 0.08)
| 2 Miscellaneous cereals       | Pasta                | S14-042869| < LOD (< 1.59)          | < LOD (< 1.72)           | 11.18          | 63.54          | < LOD (< 1.06)| < LOD (< 0.91)| 1.7 < LOD (< 7.22)|< LOD (< 0.94)| < LOD (< 11.09)| 0.03 < LOD (< 8.17)|
| 2 Miscellaneous cereals       | Pizza                | S14-042870| < LOD (< 1.38)          | < LOD (< 1.04)           | 23.50          | 66.72          | < LOD (< 1.11)| < LOD (< 0.95)| 1.7 < LOD (< 0.93)|< LOD (< 0.92)| < LOD (< 13.34)| 0.09 < LOD (< 3.98)|
| NA Group sample               | Group sample         | S14-042866| < LOD (< 2.63)          | < LOD (< 0.78)           | 37.87          | 84.45          | < LOD (< 1.09)| < LOD (< 0.85)| < LOD (< 0.85)|< LOD (< 0.33)| < LOD (< 14.97)| 0.19 < LOD (< 0.41)
| 4 Oils and fats                | Vegetable oils       | S14-042935| < LOD (< 0.92)          | < LOD (< 0.54)           | 0.68 < LOD (< 0.33)| 1.49 < LOD (< 0.05)| 65%          | 60%       | 21%        |
| 4 Oils and fats                | Fresh potatoes       | S14-042934| < LOD (< 1.00)          | < LOD (< 0.50)           | 0.68 < LOD (< 1.05)| 1.49 < LOD (< 0.05)| 65%          | 60%       | 21%        |
| 4 Oils and fats                | Potato products      | S14-042925| < LOD (< 2.44)          | < LOD (< 1.23)           | 0.60 < LOD (< 0.05)| 1.02 < LOD (< 1.11)| 77%          | 62%       | 25%        |
| NA Group sample               | Group sample         | S14-042868| < LOD (< 3.18)          | < LOD (< 0.21)           | 26.67          | 24.27          | < LOD (< 0.95)| < LOD (< 0.57)| 

**Concentrations for Deoxynivalenol repeat results are inherently corrected for recovery using an isotope labelled internal standard. Recovery values in table are for this internal standard.**
8.8. Ergot alkaloids

An established HPLC method for the analysis of ergots was transferred onto a UPLC system. The original method used a Phenomenex Gemini C\textsubscript{18} 5 \textmu m (150 x 2.1 mm) HPLC column. No UPLC column with this phase was available therefore a Waters Acquity BEH C\textsubscript{18} 1.7 \textmu m (100 x 2.1 mm) was selected because the BEH substrate is stable in high pH mobile phases. The same mobile phases as the original method were used and the gradient profile was re-optimised for the UPLC column. The method run time was shortened from 21 min to 12 min as a result of the transfer to UPLC.

Results for ergot alkaloid analysis are given in Table 22. All bread samples contained some or all of the 12 ergot alkaloids included in the analytical method. Levels found ranged from < 1 \mu g/kg to 7.51 \mu g/kg for individual ergot alkaloids in the samples. Wholemeal and granary bread contained a total of 34 \mu g/kg alkaloids. Ergot alkaloids were also detected in sandwiches at a similar level to bread samples, and at lower levels in other cereal products such as flour, breakfast cereals, biscuits and pizza as well as the group sample for these products. The alkaloids were not detected in branded food drinks, beer, cider or alternatives to milk.

8.9. Patulin

Patulin was analysed using conditions very similar to the multi- mycotoxin method; the same column and mobile phases were used and the gradient was kept the same until the patulin peak was eluted but was then rapidly increased to flush the column before re-equilibration. The injection volume was also increased because sample extracts were submitted in aqueous solvent therefore more could be injected without deterioration of the peak shape of this early-eluting compound. Carbon-13 labelled patulin internal standard was used to calculate recovery and to compensate for matrix effects.

Patulin results are presented in Table 23. Patulin was not detected in any sample. Individual LODs calculated for the samples analysed ranged from 1.7 \mu g/kg for the mushroom sample to 13.6 \mu g/kg for the group cereal sample. All samples were overspiked with \textsuperscript{13}C-patulin. Apparent patulin recovery values appeared low however the analysis was fully controlled by the use of the internal standard.
Table 22. Ergot Alkaloids Results.

<table>
<thead>
<tr>
<th>Group</th>
<th>Category</th>
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<th>LIMS Number</th>
</tr>
</thead>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 White sliced bread</td>
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<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 White unliced bread</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 Brown bread</td>
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<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4 Wholemeal and porridge</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 Other bread</td>
<td></td>
<td></td>
<td></td>
</tr>
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<td></td>
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<tr>
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<td></td>
</tr>
<tr>
<td>6 Flour</td>
<td></td>
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<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7 Buns, cakes and pastries</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8 Savoury biscuits</td>
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</tr>
<tr>
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</tr>
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</tr>
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</tr>
<tr>
<td>11 Breakfast cereals</td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>12 Rice</td>
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<td></td>
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<td></td>
</tr>
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<td>13 Other cereals products</td>
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<td>15 Pasta</td>
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<td></td>
</tr>
<tr>
<td>16 Non-alcoholic beverages</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>17 Branded food drinks</td>
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<td></td>
</tr>
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<td></td>
<td></td>
</tr>
<tr>
<td>18 Alcohol</td>
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</tr>
<tr>
<td></td>
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<td></td>
</tr>
<tr>
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</tr>
<tr>
<td></td>
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<td></td>
</tr>
<tr>
<td>20 Sambucca</td>
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</tr>
<tr>
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</tr>
</tbody>
</table>

Concentrations are corrected for recovery. Concentrations from group sample results catched using group recovery rates. ** Only one result because the category and group samples are identical.
### Table 23. Patulin Results.

<table>
<thead>
<tr>
<th>Group</th>
<th>Category</th>
<th>LIMS Number</th>
<th>Patulin Concentrations</th>
<th>Recovery</th>
</tr>
</thead>
<tbody>
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<td>1</td>
<td>Bread</td>
<td>S14-042928</td>
<td>Result</td>
<td>58%</td>
</tr>
<tr>
<td>2</td>
<td>Miscellaneous cereals</td>
<td>S14-042929</td>
<td>Result</td>
<td>15%</td>
</tr>
<tr>
<td>12</td>
<td>Potatoes</td>
<td>S14-042924</td>
<td>Result</td>
<td>20%</td>
</tr>
<tr>
<td>13</td>
<td>Other vegetables</td>
<td>S14-042925</td>
<td>Result</td>
<td>25%</td>
</tr>
<tr>
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<td>Fresh fruit</td>
<td>S14-042926</td>
<td>Result</td>
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</tr>
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<td>71</td>
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<td>S14-042927</td>
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<td>Tum, auberges</td>
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</tr>
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<td>Other fresh vegetables</td>
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<td>65%</td>
</tr>
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<td>Mushrooms</td>
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</tr>
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<td>Tomatoes</td>
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</tr>
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<td>Cucumber</td>
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<td>Dried peas</td>
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<td>72%</td>
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</tr>
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<td>S14-042944</td>
<td>Result</td>
<td>47%</td>
</tr>
<tr>
<td>93</td>
<td>Pears</td>
<td>S14-042945</td>
<td>Result</td>
<td>67%</td>
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<td>94</td>
<td>Stone fruit</td>
<td>S14-042946</td>
<td>Result</td>
<td>74%</td>
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<td>95</td>
<td>Bananas</td>
<td>S14-042947</td>
<td>Result</td>
<td>69%</td>
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<td>96</td>
<td>Grapes</td>
<td>S14-042948</td>
<td>Result</td>
<td>55%</td>
</tr>
<tr>
<td>97</td>
<td>Other fresh fruit</td>
<td>S14-042949</td>
<td>Result</td>
<td>65%</td>
</tr>
<tr>
<td>NA</td>
<td>Group sample</td>
<td>S14-042950</td>
<td>Result</td>
<td>65%</td>
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</table>

Concentrations are inherently corrected for recovery using an isotope-labelled internal standard. Recovery values in table are for this internal standard.
8.10. Sterigmatocystin

Sterigmatocystin was analysed using conditions very similar to the multi-mycotoxin method; the same column and mobile phases were used but the gradient was run in a shorter time. The injection volume was also increased because the effect on peak shape of early-eluting compounds was no longer a concern. An isotope-labelled internal standard was also added to the samples.

The sterigmatocystin results are given in Table 24. Three samples contained sterigmatocystin below the LOQ but above the LOD at levels from 0.46 to 2.17 µg/kg. These were chocolate biscuits (0.46 µg/kg), white unsliced bread (0.58 µg/kg) and herbs & spices (2.17 µg/kg). These 3 results are not quantitative as they are outside the reliable quantification range but the result is given for information as it is of value as it indicates a low level presence of this analyte. The LOQ for herbs & spices is higher due to matrix interferences seen in the chromatograms due to the large background seen in this sample. Even using the internal standard to adjust for matrix effects a higher LOQ was observed. This was a consistent pattern across all analyses of this category sample and highlights the difficulty in the analysis of these products. The LOQ is lower for the staple foods such as bread and is still acceptable for this category which forms a very minor part of the overall diet.

8.11. Citrinin

The analysis of citrinin using the multi-mycotoxin method was often unsuccessful due to the presence of interferences and also a high level of ion suppression in the MS source. It was decided that the LOD of citrinin would be improved by cleaning up extracts prior to analysis. This was achieved using new immunoaffinity columns that were a gift from R-Biopharm (Rhone).

Citrinin was analysed using the acidic multi-mycotoxin method. The injection volume was increased because the extracts had been subjected to clean-up and therefore there was less risk of simply injecting more interferences onto the column. An isotope-labelled internal standard was also added to the samples. The validation data is given in Table 25. The mean recovery for cereal spiked at 25 µg/kg was 111 % (after correction by 13C internal standard). The relative standard deviation was only 4 %, showing the method was very repeatable. Further analyses would be required to complete a full formal single laboratory validation, however the data and the use of the 13C internal standard ensure a high degree of confidence in the results found in this study.

All citrinin results are given in Table 24. All samples were below the LOQ, this ranged from 1.1 to 1.8 µg/kg, except for spices where it was slightly higher at 2.16 µg/kg. Recovery values were also very good, again the use of 13C-citrinin internal standard helped control for matrix effects. The lowest recovery was seen for
herbs & spices, where it was 46 %, highlighting the difficulty of analysing these samples.
<table>
<thead>
<tr>
<th>Group</th>
<th>Category</th>
<th>LMS Number</th>
<th>Sterigmatocystin Concentration (μg/kg)</th>
<th>Citrinin Concentration (μg/kg)</th>
<th>Cyclopiazonic Acid Concentration (μg/kg)</th>
<th>Moniliformin Concentration (μg/kg)</th>
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<tbody>
<tr>
<td>1 Bread</td>
<td>White sliced bread</td>
<td>S14-042826</td>
<td>Result: &lt; LOD (&lt; 0.24)</td>
<td>Recovery: 86%</td>
<td>&lt; LOD (&lt; 0.50)</td>
<td>Result: &lt; LOD (&lt; 1.13)</td>
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<td>White-united bread</td>
<td>S14-042827</td>
<td>Result: &lt; LOD (&lt; 0.79)</td>
<td>Recovery: 68%</td>
<td>&lt; LOD (&lt; 0.50)</td>
<td>Result: &lt; LOD (&lt; 13.34)</td>
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<td>Brown bread</td>
<td>S14-042858</td>
<td>Result: &lt; LOD (&lt; 0.28)</td>
<td>Recovery: 71%</td>
<td>&lt; LOD (&lt; 0.50)</td>
<td>Result: &lt; LOD (&lt; 23.06)</td>
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<td>Wholesome and granary bread</td>
<td>S14-042859</td>
<td>Result: &lt; LOD (&lt; 0.33)</td>
<td>Recovery: 67%</td>
<td>&lt; LOD (&lt; 0.50)</td>
<td>Result: &lt; LOD (&lt; 26.88)</td>
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<td>Other bread</td>
<td>S14-042860</td>
<td>Result: &lt; LOD (&lt; 0.25)</td>
<td>Recovery: 72%</td>
<td>&lt; LOD (&lt; 0.50)</td>
<td>Result: &lt; LOD (&lt; 18.93)</td>
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<td></td>
<td>Group sample</td>
<td>S14-042859</td>
<td>Result: 89%</td>
<td>Recovery: 76%</td>
<td>&lt; LOD (&lt; 0.50)</td>
<td>Result: &lt; LOD (&lt; 15.70)</td>
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<tr>
<td>2 Miscellaneous cereals</td>
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<td></td>
<td>Flour</td>
<td>S14-042861</td>
<td>Result: &lt; LOD (&lt; 0.27)</td>
<td>Recovery: 73%</td>
<td>&lt; LOD (&lt; 0.50)</td>
<td>Result: &lt; LOD (&lt; 15.24)</td>
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<tr>
<td></td>
<td>Buns, cakes and pastries</td>
<td>S14-042862</td>
<td>Result: &lt; LOD (&lt; 0.22)</td>
<td>Recovery: 89%</td>
<td>&lt; LOD (&lt; 0.50)</td>
<td>Result: &lt; LOD (&lt; 13.23)</td>
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<td>Savory biscuits</td>
<td>S14-042863</td>
<td>Result: &lt; LOD (&lt; 0.37)</td>
<td>Recovery: 54%</td>
<td>&lt; LOD (&lt; 0.50)</td>
<td>Result: &lt; LOD (&lt; 21.83)</td>
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<td>Sweet biscuits</td>
<td>S14-042864</td>
<td>Result: &lt; LOD (&lt; 0.22)</td>
<td>Recovery: 89%</td>
<td>&lt; LOD (&lt; 0.50)</td>
<td>Result: &lt; LOD (&lt; 19.84)</td>
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<tr>
<td></td>
<td>Chocolate biscuits</td>
<td>S14-042865</td>
<td>Result: 48 × LOD (&lt; 0.06)</td>
<td>Recovery: 85%</td>
<td>&lt; LOD (&lt; 0.50)</td>
<td>Result: &lt; LOD (&lt; 15.44)</td>
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<td>Breakfast cereals</td>
<td>S14-042866</td>
<td>Result: &lt; LOD (&lt; 0.21)</td>
<td>Recovery: 94%</td>
<td>&lt; LOD (&lt; 0.50)</td>
<td>Result: &lt; LOD (&lt; 9.35)</td>
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<td>Rice</td>
<td>S14-042867</td>
<td>Result: &lt; LOD (&lt; 0.54)</td>
<td>Recovery: 37%</td>
<td>&lt; LOD (&lt; 0.50)</td>
<td>Result: &lt; LOD (&lt; 3.97)</td>
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<tr>
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<td>Other cereal products</td>
<td>S14-042868</td>
<td>Result: &lt; LOD (&lt; 0.22)</td>
<td>Recovery: 88%</td>
<td>&lt; LOD (&lt; 0.50)</td>
<td>Result: &lt; LOD (&lt; 8.72)</td>
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<tr>
<td></td>
<td>Pasta</td>
<td>S14-042869</td>
<td>Result: &lt; LOD (&lt; 0.20)</td>
<td>Recovery: 68%</td>
<td>&lt; LOD (&lt; 0.50)</td>
<td>Result: &lt; LOD (&lt; 3.98)</td>
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<tr>
<td></td>
<td>Ground nuts including peanut butter</td>
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<td></td>
<td></td>
<td>S14-042870</td>
<td>Result: &lt; LOD (&lt; 0.15)</td>
<td>Recovery: 47%</td>
<td>&lt; LOD (&lt; 0.50)</td>
<td>Result: &lt; LOD (&lt; 21.48)</td>
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<tr>
<td></td>
<td>Other vegetables</td>
<td>S14-042871</td>
<td>Result: &lt; LOD (&lt; 0.25)</td>
<td>Recovery: 66%</td>
<td>&lt; LOD (&lt; 0.50)</td>
<td>Result: &lt; LOD (&lt; 14.19)</td>
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<tr>
<td>3 Fruit products</td>
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<td>S14-042872</td>
<td>Result: &lt; LOD (&lt; 0.15)</td>
<td>Recovery: 71%</td>
<td>&lt; LOD (&lt; 0.50)</td>
<td>Result: &lt; LOD (&lt; 25.06)</td>
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<tr>
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<td>Dried fruits</td>
<td>S14-042873</td>
<td>Result: &lt; LOD (&lt; 0.27)</td>
<td>Recovery: 72%</td>
<td>&lt; LOD (&lt; 0.50)</td>
<td>Result: &lt; LOD (&lt; 3.74)</td>
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<td>Dried spices</td>
<td>S14-042874</td>
<td>Result: &lt; LOD (&lt; 0.20)</td>
<td>Recovery: 66%</td>
<td>&lt; LOD (&lt; 0.50)</td>
<td>Result: &lt; LOD (&lt; 3.98)</td>
</tr>
<tr>
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<td>Herbs, spices</td>
<td>S14-042875</td>
<td>Result: 2 × LOD (&lt; 0.05)</td>
<td>Recovery: 44%</td>
<td>&lt; LOD (&lt; 0.50)</td>
<td>Result: &lt; LOD (&lt; 20.89)</td>
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<td>4 Non-alcoholic beverages (with added sugar)</td>
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<td>Tea</td>
<td>S14-042876</td>
<td>Result: &lt; LOD (&lt; 0.22)</td>
<td>Recovery: 91%</td>
<td>&lt; LOD (&lt; 0.50)</td>
<td>Result: &lt; LOD (&lt; 1.48)</td>
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<td>Takeaway Tea</td>
<td>S14-042877</td>
<td>Result: &lt; LOD (&lt; 0.20)</td>
<td>Recovery: 78%</td>
<td>&lt; LOD (&lt; 0.50)</td>
<td>Result: &lt; LOD (&lt; 1.35)</td>
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<td>5 Alcoholic drinks</td>
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<td>Ground coffee</td>
<td>S14-042878</td>
<td>Result: &lt; LOD (&lt; 0.20)</td>
<td>Recovery: 72%</td>
<td>&lt; LOD (&lt; 0.50)</td>
<td>Result: &lt; LOD (&lt; 2.21)</td>
</tr>
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<td>Takeaway coffee</td>
<td>S14-042879</td>
<td>Result: &lt; LOD (&lt; 0.20)</td>
<td>Recovery: 85%</td>
<td>&lt; LOD (&lt; 0.50)</td>
<td>Result: &lt; LOD (&lt; 2.88)</td>
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<td>6 Snacks</td>
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<td></td>
<td>Bread</td>
<td>S14-042880</td>
<td>Result: &lt; LOD (&lt; 0.35)</td>
<td>Recovery: 57%</td>
<td>&lt; LOD (&lt; 0.50)</td>
<td>Result: &lt; LOD (&lt; 7.84)</td>
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<tr>
<td></td>
<td>Cake</td>
<td>S14-042881</td>
<td>Result: &lt; LOD (&lt; 0.38)</td>
<td>Recovery: 68%</td>
<td>&lt; LOD (&lt; 0.50)</td>
<td>Result: &lt; LOD (&lt; 3.48)</td>
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<tr>
<td></td>
<td>Cider</td>
<td>S14-042882</td>
<td>Result: &lt; LOD (&lt; 0.38)</td>
<td>Recovery: 68%</td>
<td>&lt; LOD (&lt; 0.50)</td>
<td>Result: &lt; LOD (&lt; 3.48)</td>
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<td>White chocolate</td>
<td>S14-042883</td>
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<td>Recovery: 72%</td>
<td>&lt; LOD (&lt; 0.50)</td>
<td>Result: &lt; LOD (&lt; 3.48)</td>
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Table 25. Validation results for citrinin.

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<th>Sample</th>
<th>Citrinin</th>
<th>Citrinin</th>
<th>$^{13}$C$_{13}$-Citrinin</th>
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<tbody>
<tr>
<td></td>
<td>Conc. / µg/kg</td>
<td>Apparent Recovery (after correction by internal standard)</td>
<td>Recovery</td>
</tr>
<tr>
<td>Blank 1</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Blank 2</td>
<td>0.37</td>
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<td>81%</td>
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<tr>
<td>Spike 1 (25 µg/kg)</td>
<td>28.18</td>
<td>111%</td>
<td>66%</td>
</tr>
<tr>
<td>Spike 2 (25 µg/kg)</td>
<td>29.37</td>
<td>116%</td>
<td>58%</td>
</tr>
<tr>
<td>Spike 3 (25 µg/kg)</td>
<td>27.22</td>
<td>107%</td>
<td>54%</td>
</tr>
<tr>
<td>Spike 4 (25 µg/kg)</td>
<td>27.55</td>
<td>109%</td>
<td>57%</td>
</tr>
<tr>
<td>Spike 5 (25 µg/kg)</td>
<td>29.51</td>
<td>117%</td>
<td>55%</td>
</tr>
<tr>
<td>Spike 6 (25 µg/kg)</td>
<td>27.14</td>
<td>107%</td>
<td>52%</td>
</tr>
<tr>
<td><strong>Mean</strong></td>
<td>28.16</td>
<td>111%</td>
<td>60%</td>
</tr>
<tr>
<td><strong>SD</strong></td>
<td>1.06</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>RSD</strong></td>
<td>4%</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

8.12. Cyclopiazonic Acid

Cyclopiazonic acid is usually analysed using the acidic multi-mycotoxin method, but the peak shape was asymmetric which affected integration and sometimes also resulted in interferences co-eluting with the peak tail. The peak shape with the neutral multi-mycotoxin method deteriorated further. It was decided to analyse cyclopiazonic acid at high pH. To allow this, the column had to be changed from the Waters Acquity HSS T3 1.8 µm (100 x 2.1 mm) normally used to a Waters Acquity BEH C$_{18}$ 1.7 µm (100 x 2.1 mm) which is stable in high pH mobile phase. A simple gradient profile was used which was made steeper after the elution of cyclopiazonic acid in order to shorten the run time. An isotope-labelled internal standard was also added to the samples.

The validation data is given in Table 26. The mean recovery for cereal spiked at 25 µg/kg was 89 % (after correction by $^{13}$C internal standard). The relative standard deviation was only 4 %, showing the method was very repeatable. Further analyses would be required to complete a full formal single laboratory validation, however the data and the use of the $^{13}$C internal standard ensure a high degree of confidence in the results found in this study.

Cyclopiazonic acid results are given in Table 24. Most samples in the study were < LOD, which was set at 0.5 µg/kg. Two samples contained levels below LOQ (1.0 µg/kg), but above LOD. These were brown bread at 0.79 µg/kg and herbs and spices at 0.89 µg/kg. One sample (other snacks, not potato) contained a residue at 4.27 µg/kg. An internal standard was used for this analysis and in all cases recovery for TDS samples was in the range of 74 to 129 %. There are no limits for cyclopiazonic acid in legislation and EFSA have not evaluated it to derive a TDI. An LD$_{50}$ of 2.3 mg/kg was observed (EMAN) and the levels found here are clearly significantly below that level.
Table 26. Validation data for Cyclopiazonic acid.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Cyclopiazonic Acid</th>
<th>Cyclopiazonic Acid</th>
<th>^13C_{20}-Cyclopiazonic Acid</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Conc. / µg/kg</td>
<td>Apparent Recovery</td>
<td>Recovery</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(after correction by internal standard)</td>
<td></td>
</tr>
<tr>
<td>Blank 1</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Blank 2</td>
<td>0.00</td>
<td>97%</td>
<td>97%</td>
</tr>
<tr>
<td>Spike 1 (25 µg/kg)</td>
<td>23.01</td>
<td>92%</td>
<td>100%</td>
</tr>
<tr>
<td>Spike 2 (25 µg/kg)</td>
<td>21.63</td>
<td>87%</td>
<td>100%</td>
</tr>
<tr>
<td>Spike 3 (25 µg/kg)</td>
<td>20.90</td>
<td>84%</td>
<td>96%</td>
</tr>
<tr>
<td>Spike 4 (25 µg/kg)</td>
<td>23.10</td>
<td>92%</td>
<td>87%</td>
</tr>
<tr>
<td>Spike 5 (25 µg/kg)</td>
<td>22.57</td>
<td>90%</td>
<td>97%</td>
</tr>
<tr>
<td>Spike 6 (25 µg/kg)</td>
<td>21.76</td>
<td>87%</td>
<td>99%</td>
</tr>
<tr>
<td>Mean</td>
<td>22.16</td>
<td>89%</td>
<td>97%</td>
</tr>
<tr>
<td>SD</td>
<td>0.87</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RSD</td>
<td>4%</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

8.13. Moniliformin

Moniliformin is a very small, charged analyte which is highly soluble in water and unretained when analysed using the established multi-mycotoxin method. It elutes in the solvent front when the mobile phase is 99 % aqueous, which hinders ionisation and desolvation in the source. There is also a high possibility of co-elution with polar interferences leading to interference and ion suppression, and inaccuracy in quantification and ultimately higher LOD and LOQ. In order to improve the analytical performance and increase sensitivity a HILIC column was used to retain moniliformin in a method based on a published paper (Scarpino et al, 2013). The gradient used in this present study was steeper than that in the published method to decrease the run time. The ammonium formate buffer concentration in mobile phase A was also reduced from 100 mM to 50 mM because this resulted in a better MS response without significant deterioration of peak shape or loss of retention (reducing the concentration further resulted in a broad, early-eluting peaks). As is often the case with HILIC, a long re-equilibration time was required to obtain a stable retention time. Significant improvements were made to chromatography analytical performance. Attempts to improve the extraction and clean-up were made. Commercially available clean-up columns for moniliformin are available but by consulting literature and the manufacturer it was apparent they are only applicable for use with raw cereal flour (mainly maize), and would not be suitable for the TDS samples. Attempts were also made to source an isotopically labelled internal standard; however there are none available for moniliformin.

Other published methods used extensive concentration and blow down steps. These were mainly intended to concentrate the extracts to improve sensitivity, however as this had been achieved through the chromatography improvements by using HILIC there was no need to carry out this additional step. Based on literature and previous experience it was decided the best option would be a ‘dilute and shoot’ approach.
using the standard extraction for the multi-mycotoxin method. It was hoped this approach would lead to minimal losses as no drying step, where losses can occur, was included.

The validation data is given in Table 27. The mean recovery for cereal spiked at 25 µg/kg was 11 %. The relative standard deviation was 11 %. Therefore the method gives low but repeatable results. It is unclear why the recovery was so low, particularly for the validation samples, as the extraction solvent used was similar to others reported to give higher recovery. Previously published methods were for raw cereals, there have been no other studies of complex sample types such as those included in the TDS study.

**Table 27. Validation data for moniliformin**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Moniliformin</th>
<th>Moniliformin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Conc. / µg/kg</td>
<td>Recovery</td>
</tr>
<tr>
<td>Blank 1</td>
<td>0.08</td>
<td></td>
</tr>
<tr>
<td>Blank 2</td>
<td>0.08</td>
<td></td>
</tr>
<tr>
<td>Spike 1 (25 µg/kg)</td>
<td>1.94</td>
<td>7%</td>
</tr>
<tr>
<td>Spike 2 (25 µg/kg)</td>
<td>1.86</td>
<td>7%</td>
</tr>
<tr>
<td>Spike 3 (25 µg/kg)</td>
<td>1.85</td>
<td>7%</td>
</tr>
<tr>
<td>Spike 4 (25 µg/kg)</td>
<td>2.38</td>
<td>9%</td>
</tr>
<tr>
<td>Spike 5 (25 µg/kg)</td>
<td>1.80</td>
<td>7%</td>
</tr>
<tr>
<td>Spike 6 (25 µg/kg)</td>
<td>1.90</td>
<td>7%</td>
</tr>
<tr>
<td><strong>Mean</strong></td>
<td><strong>1.96</strong></td>
<td><strong>8%</strong></td>
</tr>
<tr>
<td><strong>SD</strong></td>
<td><strong>0.21</strong></td>
<td></td>
</tr>
<tr>
<td><strong>RSD</strong></td>
<td><strong>11%</strong></td>
<td></td>
</tr>
</tbody>
</table>

Moniliformin was not detected in any sample, full results are given in Table 24. LOQs were calculated to be from 1.3 µg/kg for takeaway tea to 39.5 µg/kg for herbs and spices. For the dried fruit sample the LOD was calculated as there was a large peak that co-eluted with moniliformin that made it difficult to accurately estimate the LOQ. Recovery values for moniliformin were extremely low, the reason for this is not known as previous work had shown the extraction method to be suitable. As the molecule is so small there is only one MRM transition that can be used for analysis. This means it is not possible to confirm the identity of the analyte using normal (triple quad) MS, which would typically be done by looking for the presence of a second and third transition and comparing the ratio of these to authentic standards. The use of Time of Flight-MS (High Resolution) was investigated, as this would allow confirmation using accurate mass, but was found to be less sensitive than LC-MS/MS and so wasn’t used for the study. It is possible that due to the improvements made to the chromatography these are ‘true’ results. Previous studies may have overestimated moniliformin as it was unretained, co-eluted with other
small molecules and without a confirmation transition it would not be possible to
discriminate between these compounds. It could simply be those samples were less
complex and suffered fewer losses during extraction. The sensitivity of the
LC-MS/MS method and the fact that every sample was overspiked at 25 µg/kg
meant that even with very low recovery reasonable LOQs could be determined, and
if moniliformin had been present in the samples it would have been detected.

9. Discussion

9.1. Calculation of Mycotoxin Levels in Group vs Category Samples.

The relative proportion of sample in each category taken to prepare a group sample
was used as the basis to calculate the expected amount of toxin present in the group
samples. This was then compared to the analytical result determined for that sample
where residues had been detected.

9.1.1. Trichothecenes

Some samples were analysed a second time as deoxynivalenol had been found but
the recovery measured was very low. The second analysis used $^{13}$C-deoxynivalenol
as an internal standard to internally correct the data. For both analyses the results of
the sum of the category samples and group samples were in excellent agreement,
both within the analysis group and between groups. For the bread category samples
there were differences between the first and second analyses of up a factor or two,
one result was approximately half the original result, two were approximately double
and 2 were in good agreement. The fact that the sum and group samples were in
good agreement would suggest that the individual differences were due to variations
in the samples due to preparation variances. For the sandwiches the category and
group sample were the same, the second analysis as the category sample gave a
slightly higher value, but the group results were the same (42.3 compared to
48.1 µg/kg).

All other results for deoxynivalenol were in good agreement. For the other
trichothecenes where residues were found (Diacetoxyscirpenol, HT-2 and T-2 toxin)
the levels were very low but were in agreement. The comparison data is presented in
Table 28.

9.1.2. Ergot alkaloids

Comparison of category and group results for ergot alkaloids are presented in Table
29. In all cases the sum of the category samples is in good agreement with the result
measured for the group sample. For the sandwiches, the category and the group
sample were the same sample therefore only one result is reported. The maximum
difference was 39 % which is within the expected variability of the analytical method.
9.1.3. Ochratoxin A

Comparison data for ochratoxin A, zearalenone, sterigmatocystin and cyclopiazonic acid is summarised in Table 30. For the bread samples, low levels (below 1 µg/kg) were found in two category samples. When the results of the other breads were summed the predicted result for the group sample was 0.13 µg/kg and a result of < 0.22 µg/kg was measured. The highest levels of ochratoxin A found were in dried fruit and fruit juices. Analyses of the other food categories in this group or the group sample itself were not requested so no direct comparison can be made. For the other groups (misc. cereals and sandwiches) results were in agreement, no ochratoxin A was found in any of the samples.

9.1.4. Zearalenone

Only 2 groups contained measurable zearalenone. For the potatoes group the result measured was in agreement with the calculated value from the category samples (< 0.66 µg/kg compared to 0.61 µg/kg). For misc. cereals the pizza sample contained ZON, at 16.45 µg/kg, and 2 other samples (sweet biscuits and chocolate biscuits) contained levels just below the LOQ. The calculated value for the group sample was 1.77 µg/kg and < 0.7 µg/kg was measured. These levels are very close or below the normal reporting limit (LOQ) of the method and the difference is within the expected variability of the method.

9.1.5. Sterigmatocystin and Cyclopiazonic Acid

Very low levels of sterigmatocystin and cyclopiazonic acid were found in a small number of samples. The calculated values for the group samples were below the method LODs and the analytical results agreed with this.
Table 28. Comparison of Category and Group Results for Trichothecenes

<table>
<thead>
<tr>
<th>Group</th>
<th>Category</th>
<th>LIMS Number</th>
<th>Proportion of category in group sample</th>
<th>Deoxynivalenol</th>
<th>Diacetoxyscirpenol</th>
<th>HT2_Toxin</th>
<th>T2_Toxin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Original Result</td>
<td>Repeat</td>
<td>LOD</td>
<td>LOD</td>
</tr>
<tr>
<td>1</td>
<td>Bread</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(&lt; 0.12)</td>
<td>(&lt; 1.41)</td>
</tr>
<tr>
<td></td>
<td>White sliced bread</td>
<td>S14-042856</td>
<td>39%</td>
<td>37.02</td>
<td>60.13</td>
<td>(&lt; LOD)</td>
<td>(&lt; 0.45)</td>
</tr>
<tr>
<td></td>
<td>White unsliced bread</td>
<td>S14-042857</td>
<td>5%</td>
<td>72.43</td>
<td>64.15</td>
<td>(&lt; LOD)</td>
<td>(&lt; 1.04)</td>
</tr>
<tr>
<td></td>
<td>Brown bread</td>
<td>S14-042858</td>
<td>6%</td>
<td>105.5</td>
<td>90.23</td>
<td>(&lt; LOD)</td>
<td>(&lt; 0.44)</td>
</tr>
<tr>
<td></td>
<td>Wholemeal and granary bread</td>
<td>S14-042859</td>
<td>21%</td>
<td>48.05</td>
<td>106.8</td>
<td>(&lt; LOD)</td>
<td>(&lt; 4.82)</td>
</tr>
<tr>
<td></td>
<td>Other bread</td>
<td>S14-042860</td>
<td>28%</td>
<td>166.4</td>
<td>78.94</td>
<td>(&lt; LOD)</td>
<td>(&lt; 0.72)</td>
</tr>
<tr>
<td></td>
<td>Group sample</td>
<td>S14-042828</td>
<td></td>
<td>77.42</td>
<td>76.73</td>
<td>(&lt; LOD)</td>
<td>(&lt; 0.46)</td>
</tr>
<tr>
<td></td>
<td>Sum of category samples</td>
<td></td>
<td></td>
<td>82.00</td>
<td>77.35</td>
<td>NA</td>
<td>0.65</td>
</tr>
<tr>
<td>2</td>
<td>Miscellaneous cereals</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(&lt; LOD)</td>
<td>(&lt; 0.10)</td>
</tr>
<tr>
<td></td>
<td>Flour</td>
<td>S14-042861</td>
<td>8%</td>
<td>27.24</td>
<td></td>
<td>(&lt; LOD)</td>
<td>(&lt; 0.12)</td>
</tr>
<tr>
<td></td>
<td>Buns, cakes and pastries</td>
<td>S14-042862</td>
<td>19%</td>
<td>10.72</td>
<td></td>
<td>(&lt; LOD)</td>
<td>(&lt; 1.42)</td>
</tr>
<tr>
<td></td>
<td>Savoury biscuits</td>
<td>S14-042863</td>
<td>2%</td>
<td>153.2</td>
<td>90.76</td>
<td>(&lt; LOD)</td>
<td>(&lt; 1.23)</td>
</tr>
<tr>
<td></td>
<td>Sweet biscuits</td>
<td>S14-042864</td>
<td>10%</td>
<td>23.75</td>
<td></td>
<td>(&lt; LOD)</td>
<td>(&lt; 4.67)</td>
</tr>
<tr>
<td></td>
<td>Chocolate biscuits</td>
<td>S14-042865</td>
<td>6%</td>
<td>17.32</td>
<td></td>
<td>(&lt; LOD)</td>
<td>(&lt; 0.43)</td>
</tr>
<tr>
<td></td>
<td>Breakfast cereals</td>
<td>S14-042866</td>
<td>17%</td>
<td>34.79</td>
<td></td>
<td>(&lt; LOD)</td>
<td>(&lt; 0.23)</td>
</tr>
<tr>
<td></td>
<td>Rice</td>
<td>S14-042867</td>
<td>11%</td>
<td>1.4 &lt; LOQ (&lt; 1.42)</td>
<td>(&lt; LOD)</td>
<td>(&lt; LOD)</td>
<td>(&lt; 0.12)</td>
</tr>
<tr>
<td></td>
<td>Other cereal products</td>
<td>S14-042868</td>
<td>6%</td>
<td>18.61</td>
<td></td>
<td>(&lt; LOD)</td>
<td>(&lt; 0.10)</td>
</tr>
<tr>
<td></td>
<td>Pasta</td>
<td>S14-042869</td>
<td>11%</td>
<td>11.18</td>
<td></td>
<td>(&lt; LOD)</td>
<td>(&lt; 0.08)</td>
</tr>
<tr>
<td></td>
<td>Pizza</td>
<td>S14-042870</td>
<td>10%</td>
<td>23.50</td>
<td></td>
<td>(&lt; LOD)</td>
<td>(&lt; 0.10)</td>
</tr>
<tr>
<td></td>
<td>Group sample</td>
<td>S14-042829</td>
<td></td>
<td>37.97</td>
<td>44.55</td>
<td>(&lt; LOD)</td>
<td>(&lt; 0.41)</td>
</tr>
<tr>
<td></td>
<td>Sum of category samples</td>
<td></td>
<td></td>
<td>21.63</td>
<td>NA</td>
<td>0.04</td>
<td>1.07</td>
</tr>
<tr>
<td>12</td>
<td>Potatoes</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(&lt; LOD)</td>
<td>(&lt; 0.16)</td>
</tr>
<tr>
<td></td>
<td>Fresh potatoes</td>
<td>S14-042924</td>
<td>68%</td>
<td>&lt; LOD (&lt; 2.60)</td>
<td>(&lt; LOD)</td>
<td>(&lt; LOD)</td>
<td>(&lt; 0.15)</td>
</tr>
<tr>
<td></td>
<td>Potato products</td>
<td>S14-042925</td>
<td>32%</td>
<td>&lt; LOD (&lt; 1.86)</td>
<td>(&lt; LOD)</td>
<td>(&lt; LOD)</td>
<td>(&lt; 0.12)</td>
</tr>
<tr>
<td></td>
<td>Group sample</td>
<td>S14-042839</td>
<td></td>
<td>&lt; LOD (&lt; 2.31)</td>
<td>(&lt; LOD)</td>
<td>(&lt; LOD)</td>
<td>(&lt; 0.12)</td>
</tr>
<tr>
<td></td>
<td>Sum of category samples</td>
<td></td>
<td></td>
<td>NA</td>
<td>NA</td>
<td>0.23</td>
<td>0.27</td>
</tr>
<tr>
<td>25</td>
<td>Sandwiches</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(&lt; LOD)</td>
<td>(&lt; 0.43)</td>
</tr>
<tr>
<td></td>
<td>Sandwiches</td>
<td>S14-042988</td>
<td>100%</td>
<td>41.65</td>
<td>60.82</td>
<td>(&lt; LOD)</td>
<td>(&lt; 0.48)</td>
</tr>
<tr>
<td></td>
<td>Group sample</td>
<td>S14-042852</td>
<td></td>
<td>42.26</td>
<td>48.06</td>
<td>(&lt; LOD)</td>
<td>(&lt; 0.43)</td>
</tr>
<tr>
<td></td>
<td>Sum of category samples</td>
<td></td>
<td></td>
<td>41.65</td>
<td>60.82</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>
Table 29. Comparison of Category and Group Results for Ergot Alkaloids

<table>
<thead>
<tr>
<th>Group</th>
<th>Category</th>
<th>LIMS Number</th>
<th>Proportion of category in group sample</th>
<th>Ergot Concentration / µg/kg</th>
<th>Concentrations are corrected for recovery. *Identity of the residue fails to confirm by ion ratio. **Only one result because the category and group samples are identical.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Bread</td>
<td>White sliced bread</td>
<td>S14-042856</td>
<td>39%</td>
<td>0.81 0.77 2.70 2.11 1.37 1.02</td>
<td></td>
</tr>
<tr>
<td>2 Bread</td>
<td>White unsoled bread</td>
<td>S14-042857</td>
<td>5%</td>
<td>0.71 0.63 1.70 1.22 0.91 0.63</td>
<td></td>
</tr>
<tr>
<td>3 Bread</td>
<td>Brown bread</td>
<td>S14-042858</td>
<td>6%</td>
<td>1.46 1.26 5.36 3.98 2.35 1.53</td>
<td></td>
</tr>
<tr>
<td>4 Bread</td>
<td>Wholemeal and granary bread</td>
<td>S14-042859</td>
<td>21%</td>
<td>1.69 1.54 7.51 5.71 2.79 2.03</td>
<td></td>
</tr>
<tr>
<td>5 Bread</td>
<td>Other bread</td>
<td>S14-042860</td>
<td>28%</td>
<td>1.32 1.16 4.08 3.05 1.96 1.27</td>
<td></td>
</tr>
<tr>
<td>NA</td>
<td>Group sample</td>
<td>S14-042828</td>
<td>1.05</td>
<td>0.91 3.63 2.77 1.54 1.05</td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>Sum of category samples</td>
<td></td>
<td></td>
<td>1.17 1.06 4.22 3.21 1.87 1.31</td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>Sandwiches</td>
<td>S14-042852</td>
<td>100%</td>
<td>0.75 0.92 1.80 2.05 1.08 1.06</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Group</th>
<th>Category</th>
<th>LIMS Number</th>
<th>Proportion of category in group sample</th>
<th>Ergot Concentration / µg/kg</th>
<th>Concentrations are corrected for recovery. *Identity of the residue fails to confirm by ion ratio. **Only one result because the category and group samples are identical.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Bread</td>
<td>White sliced bread</td>
<td>S14-042856</td>
<td>39%</td>
<td>1.31 0.95 &lt; LOQ &lt; 1.0 1.22 0.74 1.36 0.67 14.08</td>
<td></td>
</tr>
<tr>
<td>2 Bread</td>
<td>White unsoled bread</td>
<td>S14-042857</td>
<td>5%</td>
<td>1.07 0.85 &lt; LOQ &lt; 1.0 1.71 1.00 1.58 0.73 11.88</td>
<td></td>
</tr>
<tr>
<td>3 Bread</td>
<td>Brown bread</td>
<td>S14-042858</td>
<td>6%</td>
<td>2.01 1.19 2.13 1.41 3.01 1.61 27.29</td>
<td></td>
</tr>
<tr>
<td>4 Bread</td>
<td>Wholemeal and granary bread</td>
<td>S14-042859</td>
<td>21%</td>
<td>2.05 1.24 2.93 1.56 3.44 1.75 33.69</td>
<td></td>
</tr>
<tr>
<td>5 Bread</td>
<td>Other bread</td>
<td>S14-042860</td>
<td>28%</td>
<td>1.91 1.01 2.07 1.57 2.41 1.47 23.29</td>
<td></td>
</tr>
<tr>
<td>NA</td>
<td>Group sample</td>
<td>S14-042828</td>
<td>1.51</td>
<td>1.00 1.67 1.17 1.99 1.14 18.43</td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>Sum of category samples</td>
<td></td>
<td></td>
<td>1.87 0.82 1.78 1.20 2.21 1.18 21.52</td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>Sandwiches</td>
<td>S14-042852</td>
<td>100%</td>
<td>0.75 0.92 1.80 2.05 1.08 1.06</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Group</th>
<th>Category</th>
<th>LIMS Number</th>
<th>Proportion of category in group sample</th>
<th>Ergot Concentration / µg/kg</th>
<th>Concentrations are corrected for recovery. *Identity of the residue fails to confirm by ion ratio. **Only one result because the category and group samples are identical.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Bread</td>
<td>White sliced bread</td>
<td>S14-042861</td>
<td>8%</td>
<td>0.44 0.40 1.85 1.15 0.54 0.35</td>
<td></td>
</tr>
<tr>
<td>2 Bread</td>
<td>White unsoled bread</td>
<td>S14-042862</td>
<td>19%</td>
<td>0.22 &lt; LOQ &lt; 0.25 0.23 &lt; LOQ &lt; 0.25 0.32 0.33 0.22 &lt; LOQ &lt; 0.25 0.26</td>
<td></td>
</tr>
<tr>
<td>3 Bread</td>
<td>Savoury biscuits</td>
<td>S14-042863</td>
<td>2%</td>
<td>0.32 0.25 1.09 0.98 0.49 0.72</td>
<td></td>
</tr>
<tr>
<td>4 Bread</td>
<td>Sweet biscuits</td>
<td>S14-042864</td>
<td>10%</td>
<td>0.34 0.32 0.65 0.60 0.38 0.31</td>
<td></td>
</tr>
<tr>
<td>5 Bread</td>
<td>Chocolate biscuits</td>
<td>S14-042865</td>
<td>6%</td>
<td>0.15 &lt; LOQ &lt; 0.25 0.31 0.32 0.71 0.14 &lt; LOQ &lt; 0.25 0.33</td>
<td></td>
</tr>
<tr>
<td>6 Bread</td>
<td>Breakfast cereals</td>
<td>S14-042866</td>
<td>17%</td>
<td>0.45 0.63 0.53 0.63 0.47 0.53</td>
<td></td>
</tr>
<tr>
<td>7 Bread</td>
<td>Rice</td>
<td>S14-042867</td>
<td>11%</td>
<td>&lt; 0.25 &lt; 0.25 &lt; 0.25 &lt; 0.25 &lt; 0.25 &lt; 0.25</td>
<td></td>
</tr>
<tr>
<td>8 Bread</td>
<td>Other cereal products</td>
<td>S14-042868</td>
<td>6%</td>
<td>0.14 &lt; LOQ &lt; 0.25 0.14 &lt; LOQ &lt; 0.25 0.22 &lt; LOQ &lt; 0.25 0.24 &lt; LOQ &lt; 0.25 0.13 &lt; LOQ &lt; 0.25 0.16 &lt; LOQ &lt; 0.25 0.33</td>
<td></td>
</tr>
<tr>
<td>9 Bread</td>
<td>Pasta</td>
<td>S14-042869</td>
<td>11%</td>
<td>0.08 &lt; LOQ &lt; 0.25 0.03 &lt; LOQ &lt; 0.25 0.17 &lt; LOQ &lt; 0.25 0.09 &lt; LOQ &lt; 0.25 0.08 &lt; LOQ &lt; 0.25 0.05 &lt; LOQ &lt; 0.25</td>
<td></td>
</tr>
<tr>
<td>10 Bread</td>
<td>Pizza</td>
<td>S14-042870</td>
<td>10%</td>
<td>0.47 0.37 1.67 0.78 0.59 0.37</td>
<td></td>
</tr>
<tr>
<td>NA</td>
<td>Group sample</td>
<td>S14-042829</td>
<td>0.33</td>
<td>0.25 0.53 0.46 0.38 0.31</td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>Sum of category samples</td>
<td></td>
<td></td>
<td>0.20 0.24 0.51 0.48 0.23 0.27</td>
<td></td>
</tr>
<tr>
<td>13%</td>
<td>Sandwiches</td>
<td>S14-042868</td>
<td>100%</td>
<td>0.75 0.92 1.80 2.05 1.08 1.06</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Group</th>
<th>Category</th>
<th>LIMS Number</th>
<th>Proportion of category in group sample</th>
<th>Ergot Concentration / µg/kg</th>
<th>Concentrations are corrected for recovery. *Identity of the residue fails to confirm by ion ratio. **Only one result because the category and group samples are identical.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Bread</td>
<td>White sliced bread</td>
<td>S14-042861</td>
<td>8%</td>
<td>1.31 0.95 &lt; LOQ &lt; 1.0 1.22 0.74 1.36 0.67 14.08</td>
<td></td>
</tr>
<tr>
<td>2 Bread</td>
<td>White unsoled bread</td>
<td>S14-042857</td>
<td>5%</td>
<td>1.07 0.85 &lt; LOQ &lt; 1.0 1.71 1.00 1.58 0.73 11.88</td>
<td></td>
</tr>
<tr>
<td>3 Bread</td>
<td>Brown bread</td>
<td>S14-042858</td>
<td>6%</td>
<td>2.01 1.19 2.13 1.41 3.01 1.61 27.29</td>
<td></td>
</tr>
<tr>
<td>4 Bread</td>
<td>Wholemeal and granary bread</td>
<td>S14-042859</td>
<td>21%</td>
<td>2.05 1.24 2.93 1.56 3.44 1.75 33.69</td>
<td></td>
</tr>
<tr>
<td>5 Bread</td>
<td>Other bread</td>
<td>S14-042860</td>
<td>28%</td>
<td>1.91 1.01 2.07 1.57 2.41 1.47 23.29</td>
<td></td>
</tr>
<tr>
<td>NA</td>
<td>Group sample</td>
<td>S14-042828</td>
<td>1.51</td>
<td>1.00 1.67 1.17 1.99 1.14 18.43</td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>Sum of category samples</td>
<td></td>
<td></td>
<td>1.87 0.82 1.78 1.20 2.21 1.18 21.52</td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>Sandwiches</td>
<td>S14-042852</td>
<td>100%</td>
<td>0.75 0.92 1.80 2.05 1.08 1.06</td>
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</table>
Table 30. Comparison of Category and Group Results for Ochratoxin A, Zearalenone, Sterigmatocystin, and Cyclopiazonic Acid

<table>
<thead>
<tr>
<th>Group</th>
<th>Category</th>
<th>LIMS Number</th>
<th>Proportion of category in group sample</th>
<th>Ochratoxin A Concentration / µg/kg</th>
<th>Zearalenone Concentration / µg/kg</th>
<th>Sterigmatocystin Concentration / µg/kg</th>
<th>Cyclopiazonic Acid Concentration / µg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Bread</td>
<td>S14-042856</td>
<td>39%</td>
<td>&lt; LOQ (&lt; 0.22)</td>
<td>&lt; LOD (&lt; 0.34)</td>
<td>&lt; LOD (&lt; 0.24)</td>
<td>&lt; LOD (&lt; 0.50)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>S14-042857</td>
<td>5%</td>
<td>&lt; LOQ (&lt; 0.22)</td>
<td>&lt; LOD (&lt; 0.42)</td>
<td>0.58 &lt; LOQ (&lt; 0.79)</td>
<td>&lt; LOD (&lt; 0.50)</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>White sliced bread</td>
<td>S14-042858</td>
<td>6%</td>
<td>0.53</td>
<td>&lt; LOD (&lt; 0.28)</td>
<td>0.79 &lt; LOQ (&lt; 1.00)</td>
</tr>
<tr>
<td>1</td>
<td></td>
<td>S14-042859</td>
<td>21%</td>
<td>0.45</td>
<td>&lt; LOD (&lt; 0.34)</td>
<td>&lt; LOD (&lt; 0.33)</td>
<td>&lt; LOD (&lt; 0.50)</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>White unsliced bread</td>
<td>S14-042860</td>
<td>28%</td>
<td>&lt; LOQ (&lt; 0.22)</td>
<td>&lt; LOD (&lt; 0.42)</td>
<td>&lt; LOD (&lt; 0.28)</td>
</tr>
<tr>
<td>NA</td>
<td>Group sample</td>
<td>S14-042828</td>
<td></td>
<td>0.13</td>
<td>&lt; LOQ (&lt; 0.22)</td>
<td>&lt; LOD (&lt; 0.71)</td>
<td>&lt; LOD (&lt; 0.29)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>S14-042861</td>
<td>8%</td>
<td>&lt; LOQ (&lt; 0.24)</td>
<td>&lt; LOD (&lt; 0.34)</td>
<td>&lt; LOD (&lt; 0.27)</td>
<td>&lt; LOD (&lt; 0.50)</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>Flour</td>
<td>S14-042862</td>
<td>19%</td>
<td>&lt; LOQ (&lt; 0.23)</td>
<td>&lt; LOD (&lt; 0.29)</td>
<td>0.57 &lt; LOQ (&lt; 2.46)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>S14-042863</td>
<td>2%</td>
<td>&lt; LOQ (&lt; 0.26)</td>
<td>&lt; LOD (&lt; 0.29)</td>
<td>&lt; LOD (&lt; 0.37)</td>
<td>&lt; LOD (&lt; 0.50)</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>Savoury biscuits</td>
<td>S14-042864</td>
<td>10%</td>
<td>0.57 &lt; LOQ (&lt; 2.46)</td>
<td>&lt; LOD (&lt; 0.22)</td>
<td>&lt; LOD (&lt; 0.50)</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>Sweet biscuits</td>
<td>S14-042865</td>
<td>6%</td>
<td>0.85 &lt; LOQ (&lt; 2.46)</td>
<td>&lt; LOD (&lt; 0.22)</td>
<td>&lt; LOD (&lt; 0.50)</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>Chocolate biscuits</td>
<td>S14-042866</td>
<td>17%</td>
<td>0.46 &lt; LOQ (&lt; 0.66)</td>
<td>&lt; LOD (&lt; 0.22)</td>
<td>&lt; LOD (&lt; 0.50)</td>
</tr>
<tr>
<td></td>
<td>11</td>
<td>Breakfast cereals</td>
<td>S14-042867</td>
<td>11%</td>
<td>0.46 &lt; LOQ (&lt; 0.66)</td>
<td>&lt; LOD (&lt; 0.22)</td>
<td>&lt; LOD (&lt; 0.50)</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>Rice</td>
<td>S14-042868</td>
<td>6%</td>
<td>&lt; LOQ (&lt; 0.30)</td>
<td>&lt; LOD (&lt; 0.21)</td>
<td>&lt; LOD (&lt; 0.50)</td>
</tr>
<tr>
<td></td>
<td>13</td>
<td>Other cereal products</td>
<td>S14-042869</td>
<td>11%</td>
<td>&lt; LOQ (&lt; 0.30)</td>
<td>&lt; LOD (&lt; 0.21)</td>
<td>&lt; LOD (&lt; 0.50)</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>Pasta</td>
<td>S14-042869</td>
<td>10%</td>
<td>&lt; LOQ (&lt; 0.20)</td>
<td>&lt; LOD (&lt; 0.21)</td>
<td>&lt; LOD (&lt; 0.50)</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>Pizza</td>
<td>S14-042870</td>
<td>10%</td>
<td>16.45</td>
<td>&lt; LOD (&lt; 0.43)</td>
<td>&lt; LOD (&lt; 0.50)</td>
</tr>
<tr>
<td>NA</td>
<td>Group sample</td>
<td>S14-042829</td>
<td></td>
<td></td>
<td>&lt; LOQ (&lt; 0.20)</td>
<td>&lt; LOD (&lt; 0.25)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>S14-042924</td>
<td>68%</td>
<td></td>
<td>&lt; LOQ (&lt; 0.30)</td>
<td>&lt; LOD (&lt; 0.25)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>69</td>
<td>Fresh potatoes</td>
<td>S14-042925</td>
<td>32%</td>
<td>&lt; LOQ (&lt; 0.78)</td>
<td>&lt; LOD (&lt; 0.25)</td>
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<tr>
<td></td>
<td>70</td>
<td>Potato products</td>
<td>S14-042892</td>
<td></td>
<td>1.92 &lt; LOQ (&lt; 6.54)</td>
<td>&lt; LOD (&lt; 0.25)</td>
<td></td>
</tr>
<tr>
<td>NA</td>
<td>Group sample</td>
<td>S14-042839</td>
<td></td>
<td></td>
<td>&lt; LOD (&lt; 0.66)</td>
<td>&lt; LOD (&lt; 0.25)</td>
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</tr>
<tr>
<td>12</td>
<td>Potatoes</td>
<td>S14-042950</td>
<td>3%</td>
<td></td>
<td></td>
<td>&lt; LOD (&lt; 0.15)</td>
<td>&lt; LOD (&lt; 0.50)</td>
</tr>
<tr>
<td></td>
<td>98</td>
<td>Canned peaches, pears, pineapples</td>
<td>S14-042951</td>
<td></td>
<td></td>
<td></td>
<td>&lt; LOD (&lt; 0.15)</td>
</tr>
<tr>
<td></td>
<td>99</td>
<td>Other canned or frozen fruit</td>
<td>S14-042952</td>
<td>5%</td>
<td></td>
<td></td>
<td>&lt; LOD (&lt; 0.15)</td>
</tr>
<tr>
<td>16</td>
<td>Fruit products</td>
<td>S14-042953</td>
<td>86%</td>
<td></td>
<td></td>
<td></td>
<td>&lt; LOD (&lt; 0.25)</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>Dried fruit</td>
<td>S14-042954</td>
<td>6%</td>
<td>1.65</td>
<td>&lt; LOD (&lt; 0.25)</td>
<td>&lt; LOD (&lt; 0.50)</td>
</tr>
<tr>
<td></td>
<td>101</td>
<td>Fruit juices and vegetable juices</td>
<td>S14-042955</td>
<td>86%</td>
<td>5.62</td>
<td>&lt; LOD (&lt; 0.25)</td>
<td>&lt; LOD (&lt; 0.50)</td>
</tr>
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<td>NA</td>
<td>Group sample</td>
<td>S14-042843</td>
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<td></td>
<td></td>
<td></td>
<td>&lt; LOD (&lt; 0.25)</td>
</tr>
<tr>
<td>25</td>
<td>Sandwiches</td>
<td>S14-042988</td>
<td>100%</td>
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<td>&lt; LOD (&lt; 0.44)</td>
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<tr>
<td></td>
<td>138</td>
<td>Sandwiches</td>
<td>S14-042852</td>
<td>&lt; LOQ (&lt; 0.22)</td>
<td>&lt; LOD (&lt; 0.81)</td>
<td>&lt; LOD (&lt; 0.44)</td>
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</tr>
<tr>
<td>NA</td>
<td>Group sample</td>
<td>S14-042988</td>
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</tbody>
</table>
10. Conclusions

Highly sensitive LC-MS/MS-based methods for the determination of sterigmatocystin, ergot alkaloids, cyclopiazonic acid, citrinin and moniliformin in TDS samples were developed and limited validation data derived. The methods developed were then used to analyse the various TDS samples successfully.

UKAS accredited methods were used for the analysis of aflatoxins, ochratoxin A, zearalenone, patulin, fumonisins and trichothecenes. A combination of LC-MS/MS and HPLC fluorescence methods were used, chosen to give maximum sensitivity.

The most frequently detected toxins were deoxynivalenol and ergot alkaloids which were detected in all bread samples, as well as other cereal products. None of the samples exceeded any maximum permitted limit. There were very few residues of any of the other mycotoxins analysed found in the samples tested, most results were at or below the limit of quantification which were as low as technically achievable, and were typically in the sub or low µg/kg range.

Agreement between individual food category results and those of the composite group samples was on the whole very good.

This is the first UK TDS study for mycotoxins. The analysis of the individual category samples has provided additional information about these products for the first time. These results show very little incidence of mycotoxins in UK food samples, with very few results above the low limits of quantification. The data can be used for future intake calculations, to calculate background exposure to various mycotoxins from the whole diet and also to compare exposure to those calculated by other sources.
11. References

EC Directive 96/23 - Measures to monitor certain substances and residues thereof in live animals and animal products


FD Report 15/06 Total Diet Study of metals and other elements in food, Report for the UK Food Standards Agency (FS102081)

FSG 251 Determination of Aflatoxins B₁, B₂, G₁ and G₂ using Automated Immunoaffinity Column Clean-up and HPLC

FSG 252 Determination of Ochratoxin A using Automated Immunoaffinity Column Clean-up and HPLC

FSG 253 Determination of aflatoxin M₁ in milk by immunoaffinity column clean-up and HPLC

FSG 254 Determination of Patulin using HPLC or LC-MS/MS

FSG 258 Determination of Zearalenone using Immunoaffinity Column Clean-up and HPLC

FSG 261 Simultaneous Determination of Ochratoxin A and Aflatoxins B₁, B₂, G₁ and G₂ using Automated Immunoaffinity Column Clean-up and HPLC

FSG 263 Determination of a range of Trichothecenes using Solid Phase Column Clean-up and Either HPLC with Pre-column Derivatisation and Fluorescence Detection or LCMS/MS

FSG 264 Determination of Fumonisins B₁, B₂ and B₃ using Immunoaffinity Column Clean-up and Either HPLC with Pre-column Derivatisation and Fluorescence Detection or LCMS/MS

FSG 300 Analysis of Milk Products for Aflatoxin M₁

FSG 601 LC-MS/MS Method for the Determination of Ergot Alkaloids in Cereals


12. Acknowledgements

Thanks to Fera staff, Irene Leon, Joanna Stratton, Sharron Anderson, Julie Clough, Monika Sehnalova, Stephen Chapman, Paul Hepworth, Sharon Jardine, Julie Christy, Ryan Setter and Daniel Philips for their contribution to this study.
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