FOOD CHEMICAL CONTAMINANTS

Determination of Zearalenone in Barley, Maize and Wheat Flour, Polenta, and Maize-Based Baby Food by Immunoaffinity Column Cleanup with Liquid Chromatography: Interlaboratory Study

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An interlaboratory study was performed on behalf of the UK Food Standards Agency to evaluate the effectiveness of an affinity column cleanup liquid chromatography (LC) method for the determination of zearalenone (ZON) in a variety of cereals and cereal products at proposed European regulatory limits. The test portion is extracted with acetonitrile:water. The sample extract is filtered, diluted, and applied to an affinity column. The column is washed, and ZON is eluted with acetonitrile. ZON is quantified by reversed-phase LC with fluorescence detection. Barley, wheat and maize flours, polenta, and a maize-based baby food naturally contaminated, spiked, and blank (very low level) were sent to 28 collaborators in 9 European countries and 1 collaborator in New Zealand. Participants were asked to spike test portions of all samples at a ZON concentration equivalent to 100 μg/kg. Average recoveries ranged from 91–111%. Based on results for 4 artificially contaminated samples (blind duplicates) and 1 naturally contaminated sample (blind duplicate), the relative standard deviation for repeatability (RSD_r) ranged from 6.9–35.8%, and the relative standard deviation for reproducibility (RSD_R) ranged from 16.4–38.2%. The method showed acceptable within- and between-laboratory precision for all 5 matrixes, as evidenced by HorRat values <1.7.

Zearalenone [ZON; 6-(10-hydroxy-6-oxo-*trans*-1-undecenyl)-β-resorcyclic acid lactone; CAS No. 17924-92-4] is a nonsteroidal estrogenic mycotoxin produced by several *Fusarium* species. *Fusaria*, common soil fungi, are known to infect a wide variety of crops, including wheat, barley, oats, and maize.

At present, there is no legislative control in the European Union (EU) on the maximum permitted level for ZON in cereals. A temporary tolerable daily intake (t-TDI) of 0.2 μg/kg body weight/day has been established for ZON by both the European Scientific Committee on Food (SCF; 1) and the Joint Food and Agriculture Organization of the United Nations/World Health Organization (FAO/WHO) Expert Committee on Food Additives (JECFA; 2). The report of the EU Scientific Cooperation on Questions Relating to Food (SCOOP) task on the collection of occurrence data of *Fusarium* toxins has recently been published (3). Although it was concluded that the average daily intake of ZON in the adult population is less than the t-TDI value, it was suggested that harmonization in terms of sampling and analytical methodology for ZON is necessary to obtain reliable results.

Maximum limits for ZON are currently under discussion within the EU and member states. Current proposals are a limit of 200 μg/kg for unprocessed maize, maize flour, maize meal, maize grits, and refined maize oil; 100 μg/kg for unprocessed cereals other than maize; 75 μg/kg for cereal flour, except maize flour; 50 μg/kg for bread, pastries, biscuits, maize snacks, cornflakes, cereal snacks, and breakfast cereals; and 20 μg/kg for processed maize-based foods for infants and young children and other processed cereal-based foods for infants and young children and baby food (4). The European Committee for Standardization (CEN) working group on mycotoxins has identified the need for an analytical method for ZON that has been validated according to internationally recognized protocols and meets previously agreed minimum performance criteria (5). The acceptable performance characteristics for ZON are relative standard deviation for repeatability (RSD_r) ≤25%, relative standard

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Table 1. Statistical analysis of first stage trial results for zearalenone (ZON) in maize flour

Laboratory	ZON concentration, µg/kg	
	1	2
1	320.6	323.1
2	290.9	298.6
3	289.2	275.4
4	390.1	354.8
5	322.9	296.3
6	310.2	293.3
7	345.0	291.8
8	293.4	326.0
9	117.4	132.2
10	163.1	155.0
11	664.0[a]	482.0[a]
12	286.2	278.9
13	274.8	283.3
14	549.2	499.4
15	339.8	334.5
16	376.2	370.6
17	221.6	235.2
18	295.0	298.5
19	324.1	310.9
20	326.5	330.7
21	319.1	339.2
22	347.7	308.7
23	226.6	253.3
24	474.3	402.3
25	313.3	331.4
26	750.0	765.0
27	541.0	538.0
28	249.0	420.0
Matrix avg, µg/kg		335.3
Number of sets of results[b]		27
r		82.9
s_r, µg/kg		29.61
RSD_r, %		8.8
R		343.7
s_R, µg/kg		122.76
RSD_R, %		36.6
Number of outlier laboratories		1
HorRat value (HoR)		1.9

[a] Outlier results.

[b] Participant results included in the statistical analysis after noncompliant laboratories and outliers were removed.

deviation for reproducibility (RSD_R) ≤40%, and recovery of 70–100% for ZON levels of greater than 100 µg/kg.

Following from this advice, a validated method capable of supporting any regulatory limits set for ZON with the required performance criteria is required. Therefore, to support EU legislation that may be made in the future, the candidate method tested in this study targeted concentrations of ZON from 10 to 335 µg/kg.

Interlaboratory Study

Test Materials

Barley, maize and wheat flour, polenta, and maize-based baby food, both naturally contaminated and blank (very low level), were obtained from commercial sources. To achieve the target concentrations, a portion of blank sample was contaminated with a known amount of ZON solution. This was left overnight and then tumble-mixed with additional blank material to produce a homogenous sample at the desired concentration. After thorough homogenization, ca 30 g homogenized flour was packed into aluminium foil laminate sachets and heat-sealed. The samples were labelled with 2 series of numbers (not sequential) for each material to provide blind duplicates. Blank test materials of barley, maize and wheat flour, polenta, and maize-based baby food were each thoroughly homogenized before being weighed into foil sachets and sealed. These were labelled 'low level' material 1 to 5 to indicate the sample was intended for spiking. The test materials were stored at –20°C until dispatch. For distribution, the samples were packed into padded bags and sent to participants by mail. Participants acknowledged receipt of the samples upon their arrival.

Homogeneity Testing of Packaged Material

Ten samples from each batch of packaged material were removed for homogeneity testing. All samples were kept frozen at –20°C prior to homogeneity testing. The contents of each sachet were analyzed in duplicate in the Central Science Laboratory using the proposed interlaboratory trial method, but taking only half the sample size.

Data produced from the homogeneity testing were evaluated by means of analysis of variance (ANOVA; 6). From the results of the statistical evaluation, all the test materials were demonstrated to be homogenous because, in each case, the calculated *F*-value was less than the critical *F*-value. Thus, the results of the ANOVA without any outlier exclusion showed that the difference of the between-group variance and the within-group variance regarding all materials was not significant. Therefore, the between-sample SD was negligible. It was concluded that the test materials could be considered homogenous at a minimum sample intake of 12.5 g.

Organization of the Interlaboratory Study

Twenty-eight collaborators from 9 different European countries and 1 collaborator in New Zealand representing a cross section of government, food control, and food industry

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Table 2. Preparation of working calibration solutions

Working calibrant	Standard mass concentration, ng/mL	Working standard, µL	Equivalent sample concentration, µg/kg
1	150	750	2000
2	112.5	562.5	1500
3	75	375	1000
4	37.5	187.5	500
5	7.5	37.5	100

affiliations took part in the interlaboratory study. The study was carried out in 2 stages—initially only with maize flour being distributed and, after processing these results, a second stage was carried out distributing maize, wheat and barley flour, polenta, and maize-based baby food.

For the first stage, each collaborator received 1 test material of maize flour and was requested to analyze in duplicate using the prescribed method. Participants were also sent 2 blank maize flour test materials to be used for spiking and blank correction purposes. Each participant was also supplied with a set of instructions, a copy of the method to be followed, and a results reporting form. The results of the first stage of the trial for ZON in maize flour are presented in Table 1.

For the second stage of the study, which was carried out ca 11 months after the first, the same set of participants received 10 coded samples of flours (blind duplicates at 5 content levels) plus 10 labelled blank samples for spiking and blank correction purposes; a copy of the method; a set of detailed instructions; a report form for analytical data, criticisms, and suggestions; and an interlaboratory study materials receipt form. Each participant was required to prepare 1 extract from each material, perform the cleanup, and analyze the extracts by liquid chromatography (LC). Additionally participants were required to spike each of the indicated blank materials for each of the matrixes using the spiking solution (10 µg/mL ZON) prepared from the method. Participants were asked to spike a blank flour sample (as supplied) at 100 µg/kg and report the level of ZON found. Recoveries for each spiked matrix were calculated by the coordinating laboratory from results reported for spiked and nonspiked portions of the blank test materials.

Statistical Analysis of Results

The results of the 2 stages of the study were examined for evidence of individual systematic error ($p < 0.025$) using Cochran's and Grubbs' tests progressively by procedures described in the internationally agreed Protocol for the Design, Conduct, and Interpretation of Method-Performance Studies (7). Calculations for repeatability (r) and reproducibility (R) as defined by that protocol (7) were carried out on those results remaining after removal of outliers. When assessing a new method, there is often no validated reference or statutory method with which to compare precision criteria, hence, it is useful to compare the precision data obtained from an interlaboratory trial with predicted levels of precision. These predicted levels are calculated from the Horwitz equation. Comparison of the trial results and the predicted levels gives an indication as to whether the method is sufficiently precise for the level of analyte being measured (8). Historically, the Horwitz-predicted value has been calculated from the Horwitz equation (8):

$$RSD_R = 2^{(1-0.5 \log C)}$$

where C = measured concentration of analyte expressed as a decimal (e.g., 1 g/100 g = 0.01).

Thompson has recently described the use of a modified Horwitz function to predict levels of precision at µg/kg and sub-µg/kg levels (9). The use of this function was shown to give improved statistical representation levels at or below 120 µg/kg. Therefore, for the purposes of this trial, the Horwitz-predicted value was calculated from the modified Horwitz function $RSD_R = \sigma_R = 0.22c$ for values below 120 µg/kg, and from the standard Horwitz equation for those values greater than 120 µg/kg. The HorRat value (Ho_R; 10) gives a comparison of the precision measured with the precision predicted by the Horwitz equation for a method measuring at that particular level of analyte.

In the case of this trial:

$$Ho_R = RSD_R \text{ (measured)} / \sigma_R$$

An Ho_R of 1 usually indicates satisfactory interlaboratory precision, whereas a value >2 usually indicates unsatisfactory precision. Ho_R is also calculated and used to assess intralaboratory precision; for this trial:

$$RSD_r \text{ (Horwitz)} = 0.66\sigma_R$$

Experimental

(Applicable to determination of ZON at 10 to 335 µg/kg in cereals and cereal products.)

Caution: ZON is an estrogenic compound and should be treated with extreme caution. Wear gloves and safety glasses performs at all times, and, all standard and sample preparation stages in a fume hood.

Principle

Test portion is extracted with acetonitrile–water. The extract is filtered, diluted, and applied to an immunoaffinity column containing antibodies specific to ZON. ZON is removed from the immunoaffinity column with acetonitrile. ZON is separated by reversed-phase LC with fluorescence detection.

Apparatus

(**a**) *Vortex mixer.*—Or equivalent.
(**b**) *Blender or homogenizer.*

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Table 3. Interlaboratory trial results of the LC determination of ZON in maize-based baby food, barley flour, maize flour, polenta, and wheat flour[a]

	ZON concentration, µg/kg									
Lab ID	Baby food	Baby food	Barley	Barley	Maize	Maize	Polenta	Polenta	Wheat	Wheat
1	23.4	8.1	165.9	164.4	87.0	112.8	78.3	80.4	277.5	276.6
2	[b]	[b]	150.6	165.6	76.5	80.9	69.1	71.5	225.0	257.7
3	[b]	[b]	65.5	97.0	82.7	75.4	52.8	48.1	193.0	238.0
4	8.3	7.9	163.8	162.0	76.1	99.6	76.7	73.4	256.8	253.7
5	11.8	11.1	127.5	118.9	83.7	69.1	57.9	68.2	181.6	177.3
6	7.2	7.3	137.7	132.5	72.0	79.3	64.8	65.5	210.6	214.4
7	6.4	7.4	152.2	165.9	71.9	71.4	50.8	54.8	178.0	233.3
8	8.3	14.9	136.0	124.3	73.1	72.7	74.4	58.8	194.4	197.0
9	26.6[c]	19.7[c]	113.4	91.5	92.4	122.9	58.8	53.7	184.7	235.1
10	[b]	[b]	142.2	152.4	86.3	88.6	73.4	85.2	166.8	213.8
11	[b]	[b]	245.0	390.0	[b]	[b]	[b]	[b]	[b]	[b]
12	7.8	7.9	145.5	152.0	96.0	95.5	77.0	74.9	231.5	222.0
13	13.0	20.1	125.8	160.8	92.9	124.7	72.4	76.2	179.9	237.2
14	13.9	7.5	154.1	178.3	95.0	95.6	69.6	66.8	236.3	278.0
15	20.0	12.5	67.9[c]	198.1[c]	106.3	86.5	67.8	73.2	312.9	266.7
16	3.6	5.0	[b]	[b]	77.1	88.4	64.6	62.9	213.2	217.1
17	7.0	12.2	122.1	107.2	74.3	81.0	71.5	62.4	169.6	186.8
18	14.4	10.8	151.3	153.5	83.7	113.2	71.7	70.5	243.6	229.6
19	13.4	10.7	133.6	139.2	76.3	91.5	60.3	61.7	222.1	231.3
20	10.2	10.1	150.8	154.9	112.4	109.1	75.6	76.0	275.4	293.4
21	12.7	10.4	148.9	145.6	87.9	81.2	69.7	81.0	227.8	211.5
22	10.7	12.8	172.3	166.8	62.5	87.9	75.6	86.1	245.6	248.5
23	8.7	15.5	95.0	95.0	55.2	52.4	56.8	41.1	123.2	114.0
24	16.1	9.3	184.3	175.9	70.5	87.9	49.3	62.0	253.6	222.9
25	13.3	6.0	160.0	154.7	94.0	99.3	76.7	75.3	247.0	245.4
26	10.6	17.3	174.0	164.6	92.4	123.6	70.7	69.7	238.3	234.5
28	4.4	9.5	135.5	121.1	49.4	64.0	54.0	36.9	217.2	216.3
29	12.0	11.1	147.1	151.6	129.3	98.9	49.4	64.5	248.7	230.5
Mean	10.9		143.0		87.2		66.5		226.6	

[a] Outliers and noncompliant results were not included in statistical analysis; baby food, barley, maize, polenta, and wheat = samples of maize-based baby food, barley flour, maize flour, polenta, and wheat flour, respectively. Maize flour was naturally contaminated; all others were spiked samples.

[b] Noncompliant results.

[c] Outlier results.

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(**c**) *Displacement pipets.*—10, 5, and 1 mL, 250, 50, and 25 μL capacity with appropriate tips.

(**d**) *Vacuum manifold.*—To accommodate immunoaffinity columns.

(**e**) *Reservoirs and attachments.*—To fit to immunoaffinity columns.

(**f**) *Vacuum pump.*—Capable of pulling a vacuum of 10 mBar and pumping 18 L/min.

(**g**) *Filter paper.*—Whatman 113V and GF/A, or similar (Maidstone, Kent, UK).

(**h**) *LC apparatus.*—With variable injection system, a valve injection system with, e.g., a 2000 μL injection loop (for partial loop injection) and pump (isocratic, pulse-free, capable of maintaining a volume flow rate of 1 mL/min).

(**i**) *LC column.*—C_{18} reversed-phase octadecylsily (ODS) that ensures resolution of ZON from all other peaks. The maximum overlapping of peaks must be <10% (it might be necessary to adjust the mobile phase for sufficient baseline resolution). A suitable precolumn of the same composition as the main column should be used.

(**j**) *Fluorescence detector.*—Fitted with an analytical flow cell and set at 275 nm excitation wavelength and 450 nm emission wavelength.

(**k**) *UV spectrophotometer.*

(**l**) *Top pan balance.*—Accurate to 10 mg.

Reagents

All reagents are analytical grade unless otherwise stated.

(**a**) *Acetonitrile.*

(**b**) *Sodium chloride.*

(**c**) *Disodium hydrogen orthophosphate.*

(**d**) *Potassium dihydrogen phosphate.*

(**e**) *Potassium chloride.*

(**f**) *Sodium hydroxide solution (0.2 M).*—Dissolve 8 g sodium hydroxide in 1 L distilled water.

(**g**) *Phosphate buffered saline.*—Dissolve 8 g sodium chloride, 1.2 g disodium hydrogen orthophosphate, 0.2 g potassium dihydrogen phosphate, and 0.2 g potassium chloride in 1 L distilled water. Adjust the pH to 7.4 with sodium hydroxide solution (**f**).

(**h**) *Zearalenone (ZON).*—CAS No. 17924-92-4, ≥98% (Sigma-Aldrich Co. Ltd., Poole, Dorset, UK).

(**i**) *Extraction solvent.*—Water–acetonitrile (1 + 3, v/v).

(**j**) *Injection solvent for LC analysis.*—Water–acetonitrile (3 + 2, v/v).

(**k**) *Mobile phase.*—Water–acetonitrile (9 + 10, v/v); degas before use, e.g., using helium.

(**l**) *ZON stock solution (ca 10 μg/mL).*—Add 4.0 mL acetonitrile to accurately weighed ca 5 mg ZON to form a ca 1.25 mg/mL solution. Dilute 800 μL of the 1.25 mg/mL solution to 5 mL with acetonitrile to make a standard concentration of ca 200 μg/mL. Add 250 μL of the ca 200 μg/mL standard to 4.75 mL acetonitrile to form a standard concentration of ca 10 μg/mL. Calibrate the 10 μg/mL solution by recording the UV spectrum of the solution from 250 to 350 nm against the solvent used for the solution in the reference cell. Determine the concentration of the ZON solution by measuring A at the wavelength of maximum absorption close to 274 nm. The concentration of the 10 μg/mL solution will be:

$$\text{Concentration, } \mu\text{g/mL} = \frac{1000 \times A \times \text{MW}}{\varepsilon \times \delta}$$

where A = is the absorbance of the 10 μg/mL solution, MW = molecular weight of ZON (318.1), ε = molar absorptivity (12 623 for ZON in acetonitrile; 11), and δ = path length of quartz cell (cm).

(**m**) *ZON spiking solution (ca 10 μg/mL).*—Store this solution in a freezer when not in use. When stored at this temperature, the solution is stable for 2 months. Allow to reach room temperature before opening.

(**n**) *ZON working solution (1 μg/mL).*—Pipet an aliquot of solution (**m**) equivalent to 5 μg ZON into a 5 mL volumetric flask. Make up to 5 mL total volume with acetonitrile. Store this solution in a freezer when not in use. Allow to reach room temperature before opening. This solution is stable for 2 months.

(**o**) *Immunoaffinity columns (IACs).*—*See* Performance Standard for Affinity Column. For example, columns from R-Biopharm Rhone (Glasgow, UK) and Vicam (Watertown, MA) have been found to meet these criteria.

Performance Standard for Affinity Column

The IAC should contain antibodies raised against ZON. The saturation capacity of the columns should be ≥1500 ng ZON. More than 80% of ZON must be recovered when 75 ng ZON is applied in 10 mL of 15% acetonitrile in phosphate buffered saline.

Extraction

Weigh a 25 ± 0.1 g test portion of ground laboratory sample into a beaker. Add 100 mL extraction solvent and homogenize at high speed for 3 min, then filter the extract through 113 V filter paper. Take 12 mL of the filtrate and place in a conical flask with 88 mL phosphate buffered saline. If the resulting solution turns cloudy, pass through a glass fiber filter paper.

IAC Cleanup

The cleanup may be carried out by using vacuum or positive pressure, or by allowing the specified volumes to pass through the column under gravity. Do not exceed the maximum specified flow rates. Precondition the IAC with 20 mL phosphate buffered saline using a flow rate of 3–5 mL/min. Accurately measure 50 mL of the diluted sample extract and add to the reservoir. Pass the extract completely through the IAC at a rate of no more than 3 mL/min. Do not permit the IAC to run dry. Wash the IAC with 20 mL water. Dry the column by pushing ca 3 mL air through it. Place a vial under the IAC. Elute the ZON into a vial with 1.5 mL acetonitrile at a flow rate of 1–2 drops/s. Ensure that all of the elution solvent has been collected by passing 5 mL air through

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Table 4. Statistical analysis of main trial results for ZON in maize-based baby food, barley flour, maize flour, polenta, and wheat flour

Matrix (average µg/kg)	No. of sets of results[a]	r	s_r, µg/kg	RSD_r, %	R	s_R, µg/kg	RSD_R, %	No. of outlier labs	HorRat value (Ho_R)	Spike level, µg/kg	Recovery[b], %
Baby food (10.9)	23	11.0	3.9	35.8	11.7	4.2	38.2	1	1.7	100	100
Barley flour (143.0)	25	27.5	9.8	6.9	71.8	25.6	17.9	1	0.8	100	92
Maize flour (nat. contam.; 87.2)[c]	27	34.8	12.4	14.2	50.4	18.0	20.6	0	0.9	100	91
Polenta (66.5)	27	16.6	5.9	8.9	30.6	10.9	16.4	0	0.7	100	91
Wheat flour (226.6)	27	52.9	18.9	8.3	107.9	38.6	17.0	0	0.9	100	95

[a] Participant results included in the statistical analysis after noncompliant laboratories and outliers were removed.
[b] Recovery values obtained by participants' spiking low level material provided at prescribed levels.
[c] Nat. contam. = Naturally contaminated test material.

the IAC. Place the vial in a heating block at 40°C and evaporate the IAC eluate to dryness under nitrogen. Redissolve the residue in 1 mL injection solvent. Mix well using a Vortex mixer to ensure the residue is totally dissolved. Transfer to an LC vial for analysis (*V3*).

Note: The cleanup, preparation, and LC steps of this method may be carried out by an automated system such as an ASPEC (Gilson, Anachem, Luton, Beds., UK), provided that the conditions described in this method, e.g., volumes and flow rates, are adhered to.

LC Determination with Fluorescence Detection

(**a**) *LC operating conditions.*—When the column specified in *Apparatus*, (**i**), with dimensions 4.6 × 150 mm and 5 µm particle size, and the mobile phase specified in *Reagents*, (**k**) were used, the following settings were found to be appropriate: flow rate mobile phase (column), 1.0 mL/min; fluorescence detection, emission wavelength, 450 nm; fluorescence detection, excitation wavelength, 275 nm; injection volume, 400 µL.

(**b**) *Calibration graph.*—Prepare 5 LC standard solutions in separate 5 mL volumetric flasks according to Table 2. Add the appropriate amount of ZON working solution (1 µg/mL) to the volumetric flask and make up each standard to volume (5 mL) with injection solvent. Prepare a calibration graph at the beginning of every day of the analysis. Plot the equivalent concentration of ZON in the aliquot injected against peak area (or height) response.

Calculations

Determine the mass (ng) of ZON in the aliquot of test solution injected onto the LC column from the calibration graph. Calculate the mass fraction of ZON, w_{ZON}, in µg/kg using the equation:

$$w_{ZON} = m_a \times \frac{V_3}{V_4} \times \frac{V_1}{V_2} \times \frac{1000}{m_s} \times \frac{1}{1000}$$

where m_a = mass of ZON in the aliquot of test solution injected onto the column (ng); V_4 = volume of the aliquot of test solution injected onto the column (mL); V_3 = volume of the test solution (1.0 mL); V_2 = volume of sample filtrate used in cleanup (6 mL); V_1 = volume of the extraction solvent (100 mL); and ms = mass of the sample extracted (25 g). 1000 is the factor to convert g to kg; 1/1000 is the factor to convert ng to µg. Express the final result in µg/kg.

Results and Discussion

Interlaboratory Trial Results

Of the 29 laboratories that received the test samples, 28 successfully completed the study. All data submitted for the study are presented in Tables 3 and 4. The data are given as individual pairs of results for each laboratory (identified as 1 to 29). Participants spiked blank samples for each matrix with ZON at a level of 100 µg/kg. Corresponding blank samples were also analyzed unspiked. Participants were asked to report a single result each (in µg/kg) for the spiked and unspiked samples. The coordinating laboratory calculated recovery values for each participating laboratory, and the spike levels and corresponding recovery values (as means) are reported in Table 4.

Statistical Analysis of Results

Precision estimates were obtained using the 1-way ANOVA approach according to the Internatiional Union of Pure and Applied Chemistry (IUPAC) Harmonized Protocol (7). Details of the cereal matrix, average analyte concentration, RSD_r and RSD_R, number of statistical outliers and noncompliant laboratories, HorRat, and percentage recovery are presented in Table 4. The interlaboratory trial results were examined for evidence of individual systematic error ($p < 0.025$) using Cochran's, and Grubbs' tests progressively (7). Pairs of results that were identified as outliers are indicated in Table 3. Noncompliant results were identified as those for which no statistics were possible, such as single results instead of pairs of results or "less than" values

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instead of numerical results being reported. For the results given in Table 3, the maximum number of outliers identified was 1 laboratory, giving acceptable data ranging from 27 to 28 laboratories.

Comments from Interlaboratory Trial Participants

The study was carried out in 2 stages. Initially only maize flour was analyzed, and comments and results were received. Subsequently, the second part of the study involved other cereals and a cereal-based baby food (barley flour, maize flour, wheat flour, polenta, and maize-based baby food), and again comments were invited. Following the first stage of the trial, the method was modified slightly to take into account comments made by participants regarding preparation of calibration standards, preparation of the test solution after elution from the IAC, and calculation used for obtaining results. No problems were reported in the use of the method. From information supplied on the results reporting form, it was found that participants used 2 different types of commercial IACs and a range of LC columns. However, most participants used the same chromatography conditions, although some modified conditions slightly to achieve the desired performance. All participants were asked to include their chromatograms when returning their trial results. The coordinating laboratory assessed all of the chromatograms provided as satisfactory.

Precision Characteristics of the Method

No participating laboratories were found to be noncompliant by the coordinating laboratory in the first stage of the trial. Failure to return results, procedural errors, and failure to report duplicate results constituted noncompliant data. These results, if reported, were excluded from the calculation of performance criteria. One laboratory that participated in the first stage of the trial did not continue to participate in the main trial.

The precision data for all samples in the main trial are summarized in Table 4. Based on results for the naturally contaminated maize flour sample (blind duplicate) used in the main trial, RSD_r was 14.2% at a level of 87 μg/kg and the RSD_R was 20.6%. Based on results for fortified samples (blind pairs) used in the main trial, the RSD_r ranged from 6.9–35.8% and the RSD_R from 16.4–38.2%. The average recovery of ZON derived from the cereal samples spiked by participants ranged from 91–100%.

Interpretation of Results

The acceptability of the precision characteristics of the method were assessed on the basis of the Ho_R (10), which compare the RSD_R at the various levels with those values predicted from interlaboratory trial studies taken from the published literature. The precision values for all matrixes were well within the satisfactory limits derived by the Horwitz equation (i.e., <2.0). The Ho_R ranged from 0.7 to 1.7, with 4 out of 6 being below or equal to 1.0. The matrix with the highest Ho_R was a maize-based baby food. This was to be expected because the concentration of ZON determined in this matrix was close to the quantification limit of the method, and the matrix itself was more complex than the other flour-based samples.

Sensitivity

The satisfactory Ho_R of 1.7 obtained for the baby food sample, which contained a mean level of 10.9 μg/kg, demonstrates that the method is applicable and reliable at this level.

The method has the advantage of being quick and easy to use, and is easily transferable to routine and control laboratories because it avoids the use of specialized equipment. It has proved to be robust under the conditions of the interlaboratory study, and applicable across a range of cereal matrixes that are likely to be contaminated with ZON.

Conclusions

This paper reports the first interlaboratory study of an IAC method for ZON in cereals and cereal products. The method, involving IAC cleanup and determination by reversed-phase LC, has been successfully validated at ≥ 10 to ≤ 335 μg/kg for a variety of cereal matrixes. The method is suitable for enforcement purposes to test compliance with proposed European Directives; it has been shown to have performance characteristics that fulfill European requirements (5) and justify putting the method forward for consideration as a CEN standard and as a candidate for an AOAC First Action method.

This study forms part of the Food Standards Agency Collaborative Trial Program. In addition to producing validated methods that can be used in the UK and by the European Commission for enforcement purposes, the Program also addresses wider measurement issues.

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