



Fapas[®] – Food Chemistry Proficiency Test Report 02486

Nitrofurans Metabolites in Prawns

October-November 2022

PARTICIPANT LABORATORY NUMBER

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Laboratory numbers are displayed in SecureWeb next to the download link for this report.

REPORT INTEGRITY

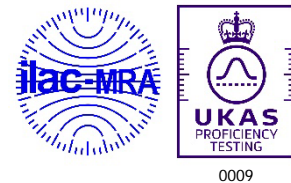
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SUMMARY

1. The test materials for Fapas[®] – Food Chemistry proficiency test 02486 were dispatched in October 2022. Each participant received a prawns test material to be analysed for a selection of nitrofurans metabolites.
2. An assigned value (x_a) was determined for each analyte and in conjunction with the standard deviation for proficiency (σ_p) was used to calculate a z-score for each result. However, it was not possible to set an assigned value for AHD (bound), AOZ (bound), and SEM (bound).
3. Results for this proficiency test are summarised as follows:

analyte	assigned value, x_a	units	number of scores, $ z \leq 2$	total number of scores	% $ z \leq 2$
AHD (bound)	not set				
AHD (total)	1.66	$\mu\text{g}/\text{kg}$	34	44	77
AOZ (bound)	not set				
AOZ (total)	1.76	$\mu\text{g}/\text{kg}$	44	50	88
SEM (bound)	not set				
SEM (total)	3.30	$\mu\text{g}/\text{kg}$	34	46	74
Total Nitrofurans Metabolites	6.89	$\mu\text{g}/\text{kg}$	11	16	69

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1. INTRODUCTION

1.1. Proficiency Testing

Proficiency testing aims to provide an independent assessment of the competence of participating laboratories. Together with the use of validated methods, proficiency testing is an essential element of laboratory quality assurance.

Further details of the Fapas[®] – Food Chemistry proficiency testing scheme are available in our protocols [7, 8].

2. TEST MATERIAL

2.1. Preparation

Preparation of the samples for this proficiency test was sub-contracted to a laboratory meeting the quality requirements of the scheme's accreditation [3].

The test materials were prepared from Whiteleg shrimp (*Litopenaeus vannamei*).

AHD, AOZ and SEM were spiked into the test material.

Samples were stored at -20°C until dispatch.

2.2. Screening and Homogeneity

To test for homogeneity, randomly selected test materials were analysed in duplicate. Testing was sub-contracted to a laboratory meeting the quality requirements of the scheme's accreditation [3].

These data showed sufficient homogeneity and were not included in the subsequent calculation of the assigned values.

At the same time, samples were screened for the presence of other nitrofurans metabolites, see Section 3 for the list. No other residues were detected at or above the testing laboratory's reporting limit.

2.3. Dispatch

The start date was 24 October 2022. Test materials were sent to 62 participants.

3. RESULTS

The instructions for reporting results were as follows:

- 1) Identify and determine the level of analyte(s) present in the test material as follows:

analyte	units	comment
AHD (bound)	µg/kg	(1-aminohydantoin), the metabolite of nitrofurantoin, corrected for recovery
AHD (total)	µg/kg	(1-aminohydantoin), the metabolite of nitrofurantoin, corrected for recovery

analyte	units	comment
AMAZ (bound)	µg/kg	(3-amino-5-methylmorpholino-2-oxazolidinone) the metabolite of furaltadone, corrected for recovery
AMAZ (total)	µg/kg	(3-amino-5-methylmorpholino-2-oxazolidinone) the metabolite of furaltadone, corrected for recovery
AZ (bound)	µg/kg	(3-amino-2-oxazolidinone), the metabolite of furazolidone, corrected for recovery
AZ (total)	µg/kg	(3-amino-2-oxazolidinone), the metabolite of furazolidone, corrected for recovery
SEM (bound)	µg/kg	(semicarbazide) the metabolite of nitrofurazone, corrected for recovery
SEM (total)	µg/kg	(semicarbazide) the metabolite of nitrofurazone, corrected for recovery
Total nitrofurans metabolites	µg/kg	for ELISA kit users (see point 6 below), sum of all nitrofurans metabolites present, corrected for recovery

PLEASE NOTE: Not all residues will be present. It is important that you report the results in this way so that we can include as many results as possible in the statistical analysis.

2) Participants may submit results for the nitrofurans metabolites in two forms:

- As bound: a procedure which usually requires a pre-washing step.
- And/or as total: the sum of bound plus free nitrofurans metabolites.

Please note that, in previous proficiency tests we have received low numbers of submitted results for bound metabolites and we may not be able to set an assigned value. Therefore, if you analyse for bound residues, you are recommended to analyse for total as well.

3) All residues are to be reported as specified above. If this is not possible, use the comments box to note any residues that are *not* reported in the form specified.

- For each residue, select either "Not Detected", "Not Tested" or "Provide Result".
- Enter a default value for "% recovery" and "Reporting Limit µg/kg".
- AFTER you have entered your results for each residue you MUST review and if necessary, edit the values for "% recovery" and "Reporting Limit µg/kg" that differ from the default value you gave.

4) In the Internal Standard/Recovery Correction column on the results form:

- Enter 'Y' if you added an internal standard at the start,
- Enter your % recovery if this was measured,
- Enter 'M' if you used a matrix-extracted calibration curve,
- Enter 'S' if you used standard addition.

5) Please state a Reporting Limit for each analyte or marker residue included in your assay. This can be either CC β (for those laboratories operating to EC 657/2002 guidelines) or the Limit of Quantification (LoQ). The Reporting Limit value will be used in our assessment of false negatives for any undetected analytes included in your assay.

6) The analyte for total nitrofurans metabolites is intended for those laboratories using ELISA kits which don't differentiate between different veterinary medicines that correspond to a particular chemical class. Such ELISA kits will usually only report the

total sum of that chemical class. Participants using chromatographic methods are welcome to report the total (sum of individual residues) of that chemical class as well.

- 7) This is an identification and quantification proficiency test. Therefore, if you analyse for a residue that is in the test material, and do not identify it, and your reporting limit is below the level needed for a z-score of -3.0, you will be assessed as if your result was zero.

Results were submitted by 54 participants (87%) before the closing date for this test, 25 November 2022.

Each participant was given a laboratory number, assigned in order of receipt of results. The reported analyte concentrations are given in Table 1 to Table 3.

If a participant analysed for a residue that was in the test material, but did not identify it, and their reporting limit was below the level needed for a z-score of -3.0 or was unstated, they were assessed as if their result was zero.

If a participant analysed for a residue that was in the test material, but did not identify it, and their reporting limit was above the level needed for a z-score of -3.0, the result was recorded as <RL.

Any participant identifying residues other than AHD, AOZ and SEM are listed in Table 4 together with the recovery information and reporting limit.

Participants' comments are given in Table 5.

The analytical methods used by each participant are summarised in APPENDIX I.

4. STATISTICAL EVALUATION OF RESULTS

The results submitted by participants were statistically analysed in order to provide an assigned value for each analyte. The assigned values were then used in combination with the standard deviation for proficiency, σ_p , to calculate a z-score [9] for each result. The procedure is detailed in the relevant protocols [7, 8].

Further background on the procedure followed can be found in the IUPAC International Harmonised Protocol for the Proficiency Testing of Analytical Chemistry Laboratories [10].

4.1. Calculation of the Assigned Value, x_a

The assigned value, x_a , for each analyte was derived from the consensus of the results submitted by participants.

The procedure used to derive this consensus involved:

- i) exclusion, if present, of any non numerical results i.e. qualitative or semi-quantitative results,
- ii) exclusion, if present, of any results that were approximately 10, 100 or 1000 × greater or smaller than the majority of submitted results (as these were considered to be reporting errors),
- iii) exclusion of results where use of neither a matrix-extracted calibration curve, nor a recovery %, nor standard addition was reported (as these results were considered to be uncorrected for recovery).

For AOZ (total), this procedure was straightforward and the robust mean was chosen as the assigned value.

For AHD (total) and SEM (total), the mode was chosen as the assigned value because the distribution of results was skewed. Kernel density plots of the distributions can be seen in Figure 2 and Figure 6.

For total nitrofurans metabolites, the median was chosen as the assigned value because of the low number of results submitted.

For AHD (bound), AOZ (bound) and SEM (bound) it was not possible to set an assigned value due to the high associated uncertainties. Results are shown in Tables 1-3 and in Figures 1, 3 and 5 for comparison.

The assigned values for all analytes are shown in Table 6.

4.2. Standard Deviation for Proficiency, σ_p

The standard deviation for proficiency, σ_p , was set at a value that reflects best practice for the analyses in question.

For all analytes, σ_p was derived from the appropriate form of the Horwitz equation [11].

The values for σ_p used to calculate z-scores from the reported results of this test are given in Table 6.

4.3. Individual z-Scores

Participants' z-scores were calculated as:

$$z = \frac{(x - x_a)}{\sigma_p}$$

where x = the participant's reported result,

x_a = the assigned value, see Table 6,

and σ_p = the standard deviation for proficiency, see Table 6.

Participants' z-scores for all analytes are given in Table 1 to Table 3 and shown as histograms in Figures 2, 4, 6 and 7. It is possible for the z-scores published in this report to differ slightly from the z-score that can be calculated using the formula given above. These differences arise from the necessary rounding of the actual assigned values and standard deviations for proficiency prior to their publication in Table 6.

The number and percentage of z-scores in the range $-2 \leq z \leq 2$ for all analytes are given in Table 7.

5. INTERPRETATION OF SCORES

In normal circumstances, over time, about 95% of z-scores will lie in the range $-2 \leq z \leq 2$. Occasional scores in the range $2 < |z| < 3$ are to be expected, at a rate of 1 in 20. Whether or not such scores are of importance can only be decided by considering them in the context of the other scores obtained by that laboratory.

Scores where $|z| > 3$ are to be expected at a rate of about 1 in 300. Given this rarity, such z-scores very strongly indicate that the result is not fit-for-purpose and almost certainly requires investigation.

The consideration of a set or sequence of z-scores over time provides more useful information than a single z-score. Examples of suitable methods of comparison are provided in the IUPAC

International Harmonised Protocol for the Proficiency Testing of Analytical Chemistry Laboratories [10].

6. REFERENCES

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- 2 GlobalSign AATL Document Signing FAQs, <https://support.globalsign.com/aatl-document/aatl-document-signing-faqs> accessed 24/03/2022.
- 3 ISO/IEC 17043:2010, Conformity assessment – General requirements for proficiency testing.
- 4 The ILAC Mutual Recognition Arrangement, <https://ilac.org/ilac-mra-and-signatories/> accessed 24/03/2022.
- 5 Fera Science Ltd, Standards & Accreditations, <https://www.fera.co.uk/about-us/standards-and-accreditation> accessed 24/03/2022.
- 6 Lloyd's Register, Learn about ISO 9001 Quality Management Systems (QMS), <https://www.lr.org/en-gb/iso-9001/> accessed 24/03/2022.
- 7 Fapas[®], 2021, Protocol for Proficiency Testing Schemes, Version 7, January 2021, Part 1 – Common Principles.
- 8 Fapas[®], 2017, Protocol for Proficiency Testing Schemes, Version 5, April 2017, Part 2 – Fapas[®] Food Chemistry scheme (FAPAS).
- 9 AMC Tech Brief No. 74, z-Scores and other scores in chemical proficiency testing – their meanings, and some common misconceptions, *Anal. Methods*, 2016, **8**, 5553.
- 10 Thompson, M., Ellison, S.L.R. and Wood, R., 2006, The International Harmonised Protocol for the Proficiency Testing of Analytical Chemistry Laboratories, *Pure Appl. Chem.*, **78**, No. 1, 145–196.
- 11 Thompson, M., 2000, Recent trends in inter-laboratory precision at ppb and sub-ppb concentrations in relation to fitness for purpose criteria in proficiency testing, *Analyst*, **125**, 385-386.

Table 1: Results and z-Scores for AHD (bound) and AHD (total)

laboratory number	analyte							
	AHD (bound) assigned value: not set				AHD (total) assigned value: 1.66 µg/kg			
	result, µg/kg	IS/% rec	RL	z-score	result	IS/% rec	RL	z-score
001	#				1.61	Y/M	0.40	-0.1
002	0.01	Y	Åµg/kg		2.33	Y	Åµg/kg	1.8
003	#				1.952	80-120%	0.2	0.8
004	#				1.49	Y	0.25	-0.5
005	1.45	Y	0.5		#			
006	#				1.7	Y	1	0.1
007	#				1.76	Y	0.5	0.3
008	#				1.77	Y % M S	0.5	0.3
009	#				2	Y	0.059	0.9
010	#				1.32	0.0	0.5	-0.9
011	#				#			
012	#				1.95	Y	0.5	0.8
013	#				1.77	y / m	1	0.3
014	#				1.58	Y/89.66%/M	1	-0.2
015	#				1.91	Y	0.10	0.7

RL = reporting limit # = not analysed Y = internal standard used M = matrix-extracted calibration S = standard addition
z-scores outside $|z| > 2$ are shown in **bold**, see Section 5

Table 1 (continued): Results and z-Scores for AHD (bound) and AHD (total)

laboratory number	analyte							
	AHD (bound) assigned value: not set				AHD (total) assigned value: 1.66 µg/kg			
	result, µg/kg	IS/% rec	RL	z-score	result	IS/% rec	RL	z-score
016	#				1.88			0.6
017	#				0	Y, M	0.1	-4.5
018	#				#			
019	0.75	Y, 108%, M	0.5		1.9	Y, 104%, M	0.5	0.6
020	#				<RL		1	
021	#				1.88		0.5	0.6
022	#				2.1	Y	1	1.2
023	not detected				0.47	112%		-3.3
024	0.36		0.2		1.57	87.3%	0.2	-0.3
025	#				1.60	YS	0.5	-0.2
026	0.04	Y			1.72	Y		0.2
027	#				2.37	Y	1	1.9
028	#				3.02	96%	0.0375	3.7
029	#				1.60	Y	1	-0.2
030	#				0.34		0.05	-3.6

RL = reporting limit # = not analysed Y = internal standard used M = matrix-extracted calibration S = standard addition
z-scores outside $|z| > 2$ are shown in **bold**, see Section 5

Table 1 (continued): Results and z-Scores for AHD (bound) and AHD (total)

laboratory number	analyte							
	AHD (bound) assigned value: not set				AHD (total) assigned value: 1.66 µg/kg			
	result, µg/kg	IS/% rec	RL	z-score	result	IS/% rec	RL	z-score
031	#				1.53	Y	0.2	-0.4
032	not detected		0.5		1.75		0.5	0.2
033	#				1.45	Y	0.5	-0.6
034	#				1.54	Y	0.5	-0.3
035	#				#			
036	#				1.83	Y	0.1	0.5
037	#				#			
038	#				1.43	83.10	1	-0.6
039	#				1.7		0.05	0.1
040	2.91	Y	0.141		#			
041	not detected	recovery correction			1.36	recovery correction	97.8	-0.8
042	#				1.65	Y	0.5	0.0
043	#				0	Y	0.5	-4.5
044	#				1.82		0.2	0.4
045	#				3.10	Y,M	0.25	3.9

RL = reporting limit # = not analysed Y = internal standard used M = matrix-extracted calibration S = standard addition
z-scores outside $|z| > 2$ are shown in **bold**, see Section 5

Table 1 (continued): Results and z-Scores for AHD (bound) and AHD (total)

laboratory number	analyte							
	AHD (bound) assigned value: not set				AHD (total) assigned value: 1.66 µg/kg			
	result, µg/kg	IS/% rec	RL	z-score	result	IS/% rec	RL	z-score
046	#				> 0.25	YES	0.25	
047	#				1.41	Yes/No	0.10	-0.7
048	not detected				1.5	Y	0.6	-0.4
049	#				#			
050	#				2.70		0.25	2.8
051	#				#			
052	#				2.84	Y	0.5	3.2
053	#				2.46	Y	0.5	2.2
054	#				2.9	YES	0.13	3.4

RL = reporting limit # = not analysed Y = internal standard used M = matrix-extracted calibration S = standard addition
z-scores outside $|z| > 2$ are shown in **bold**, see Section

Table 2: Results and z-Scores for AOZ (bound) and AOZ (total)

laboratory number	analyte							
	AOZ (bound) assigned value: not set				AOZ (total) assigned value: 1.76 µg/kg			
	result, µg/kg	IS/% rec	RL	z-score	result	IS/% rec	RL	z-score
001	#				1.83	Y/M	0.40	0.2
002	0.05	Y	Åµg/kg		2.25	Y	Åµg/kg	1.3
003	#				1.207	80-120%	0.05	-1.4
004	#				1.47	Y	0.25	-0.7
005	2.01	Y	0.5		#			
006	#				1.7	Y	1	-0.2
007	#				1.49	Y	0.5	-0.7
008	#				1.74	Y % M S	0.5	0.0
009	#				2.147	Y	0.057	1.0
010	#				1.71	0.0	0.5	-0.1
011	#				1.8	Y	0.5	0.1
012	#				3.95	Y	0.2	5.7
013	#				1.84	y / m	1	0.2
014	#				1.72	Y/99.52%/M	0.5	-0.1
015	#				1.97	Y	0.10	0.5

RL = reporting limit # = not analysed Y = internal standard used M = matrix-extracted calibration S = standard addition
z-scores outside |z| >2 are shown in bold, see Section

Table 2 (continued): Results and z-Scores for AOZ (bound) and AOZ (total)

laboratory number	analyte							
	AOZ (bound) assigned value: not set				AOZ (total) assigned value: 1.76 µg/kg			
	result, µg/kg	IS/% rec	RL	z-score	result	IS/% rec	RL	z-score
016	#				1.54			-0.6
017	#				1.48	Y, M	0.1	-0.7
018	#				1.18	98.4	0.16	-1.5
019	0.78	Y, 99%, M	0.5		2.3	Y, 99%, M	0.5	1.4
020	#				15.81		1	36.3
021	#				1.69		0.5	-0.2
022	#				1.98	Y	1	0.6
023	not detected				0.62	105%		-2.9
024	0.53		0.2		1.88	101.9%	0.2	0.3
025	#				1.75	YS	0.5	0.0
026	0.03	Y			1.62	Y		-0.4
027	#				1.74	Y	1	0.0
028	#				2.63	100%	0.018	2.3
029	#				1.83	Y	1	0.2
030	#				5.40		0.05	9.4

RL = reporting limit # = not analysed Y = internal standard used M = matrix-extracted calibration S = standard addition
z-scores outside $|z| > 2$ are shown in bold, see Section 5

Table 2 (continued): Results and z-Scores for AOZ (bound) and AOZ (total)

laboratory number	analyte							
	AOZ (bound) assigned value: not set				AOZ (total) assigned value: 1.76 µg/kg			
	result, µg/kg	IS/% rec	RL	z-score	result	IS/% rec	RL	z-score
031	#				1.44	Y	0.1	-0.8
032	not detected		0.5		1.58		0.5	-0.5
033	#				1.56	Y	0.5	-0.5
034	#				1.69	Y	0.5	-0.2
035	#					AMOZ - 0.0613 ppb AOZ - 0.00654 ppb	AMOZ - 0.143 ppb AOZ - 0.146 ppb	
036	#				1.83	Y	0.1	0.2
037	#				1.12	Y %	1.12	-1.7
038	#				1.90	86.73	1	0.4
039	#				1.8		0.05	0.1
040	2.01	Y	0.138		#			
041	not detected	recovery correction			2.46	recovery correction	97.0	1.8
042	#				1.68	Y	0.5	-0.2
043	#				0.41	Y,M	0.25	-3.5
044	#				1.55		0.2	-0.5
045	#				2.07	Y,M	0.25	0.8

RL = reporting limit # = not analysed Y = internal standard used M = matrix-extracted calibration S = standard addition
z-scores outside $|z| > 2$ are shown in **bold**, see Section 5

Table 2 (continued): Results and z-Scores for AOZ (bound) and AOZ (total)

laboratory number	analyte							
	AOZ (bound) assigned value: not set				AOZ (total) assigned value: 1.76 µg/kg			
	result, µg/kg	IS/% rec	RL	z-score	result	IS/% rec	RL	z-score
046	#				> 0.25	YES	0.25	
047	#				1.72	Yes/No	0.10	-0.1
048	not detected				1.8	Y	0.5	0.1
049	#				1.5	Y	0.2	-0.7
050	#				1.75		0.25	0.0
051	#				1.767	Y	0.8	0.0
052	#				1.47	Y	0.5	-0.7
053	#				2.03	Y	0.5	0.7
054	#				2.18	YES	0.13	1.1

RL = reporting limit # = not analysed Y = internal standard used M = matrix-extracted calibration S = standard addition
z-scores outside $|z| > 2$ are shown in **bold**, see Section 5

Table 3: Results and z-Scores for SEM (bound), SEM (total) and Total Nitrofurans Metabolites

laboratory number	analyte											
	SEM (bound) assigned value: not set				SEM (total) assigned value: 3.30 µg/kg				Total Nitrofurans Metabolites assigned value: 6.89 µg/kg			
	result, µg/kg	IS/% rec	RL	z-score	result	IS/% rec	RL	z-score	result	IS/% rec	RL	z-score
001	#				4.15	Y/M	0.40	1.2	#			
002	0.16	Y	Âµg/kg		4.28	Y	Âµg/kg	1.3	#			
003	#				2.621	80-120%	0.1	-0.9	#			
004	#				3.10	Y	0.25	-0.3	6.06	Y	0.25	-0.5
005	3.07	Y	0.5		#				0		0.5	-4.5
006	#				#				#			
007	#				4.33	Y	0.5	1.4	#			
008	#				3.55	Y % M S	0.5	0.3	7.06	Y % M S	0.5	0.1
009	0.189	Y	0.089		4.01	Y	0.069	1.0	#			
010	#				2.88	0.0	0.5	-0.6	#			
011	#				4.2	Y	0.5	1.2	#			
012	#				1.80	Y	0.5	-2.1	#			
013	#				3.58	y / m	1	0.4	#			
014	#				3.15	Y/86.16%/ M	1	-0.2	#			
015	#				3.01	Y	0.10	-0.4	6.89	Y	0.10	0.0

RL = reporting limit # = not analysed Y = internal standard used M = matrix-extracted calibration S = standard addition
z-scores outside |z| > 2 are shown in **bold**, see Section 5

Table 3 (continued): Results and z-Scores for SEM (bound), SEM (total) and Total Nitrofurans Metabolites

laboratory number	analyte											
	SEM (bound) assigned value: not set				SEM (total) assigned value: 3.30 µg/kg				Total Nitrofurans Metabolites assigned value: 6.89 µg/kg			
	result, µg/kg	IS/% rec	RL	z-score	result	IS/% rec	RL	z-score	result	IS/% rec	RL	z-score
016	#				3.93			0.9	#			
017	#				1.52	Y, M	0.1	-2.5	#			
018	#				#				#			
019	1.5	Y, 90%, M	0.5		5.3	Y, 92%, M	0.5	2.7	9.5		0.5	1.7
020	#				7.79		1	6.2	0		1	-4.5
021	#				3.51		0.5	0.3	#			
022	#				3.61	Y	1	0.4	#			
023	not detected				1.04	76%		-3.1	3.24			-2.4
024	0.85		0.2		3.36	97.6%	0.2	0.1	6.81		0.2	-0.1
025	#				2.87	YS	0.5	-0.6	#			
026	0.23	Y			3.37	Y		0.1	#			
027	#				5.47	Y	1	3.0	#			
028	#				5.54	99.1%	0.0375	3.1	#			
029	#				2.84	Y	1	-0.6	#			
030	#				2.63		0.05	-0.9	8.03			0.8

RL = reporting limit # = not analysed Y = internal standard used M = matrix-extracted calibration S = standard addition
z-scores outside $|z| > 2$ are shown in **bold**, see Section 5

Table 3 (continued): Results and z-Scores for SEM (bound), SEM (total) and Total Nitrofurans Metabolites

laboratory number	analyte											
	SEM (bound) assigned value: not set				SEM (total) assigned value: 3.30 µg/kg				Total Nitrofurans Metabolites assigned value: 6.89 µg/kg			
	result, µg/kg	IS/% rec	RL	z-score	result	IS/% rec	RL	z-score	result	IS/% rec	RL	z-score
031	#				2.89	Y	0.2	-0.6	#			
032	not detected		0.5		3.36		0.5	0.1	6.69		0.5	-0.1
033	#				3.16	Y	0.5	-0.2	#			
034	#				3.19	Y	0.5	-0.2	#			
035	#				#				#			
036	#				3.73	Y	0.1	0.6	7.39	Y	0.25	0.3
037	#				#				#			
038	#				3.17	85.10	1	-0.2	#			
039	#				3.4		0.05	0.1	6.9		0.05	0.0
040	not detected	Y			#				#			
041	not detected	recovery correction			3.09	recovery correction	91.2	-0.3	0	recovery correction		-4.5
042	#				2.94	Y; 111%	0.5	-0.5	#			
043	#				1.26	Y,M	0.5	-2.8	1.67	Y,M	0.25	-3.4
044	#				4.54		0.2	1.7	#			
045	#				5.08	Y,M	0.25	2.4	#			

RL = reporting limit # = not analysed Y = internal standard used M = matrix-extracted calibration S = standard addition
z-scores outside |z| >2 are shown in **bold**, see Section 5

Table 3 (continued): Results and z-Scores for SEM (bound), SEM (total) and Total Nitrofurans Metabolites

laboratory number	analyte											
	SEM (bound) assigned value: not set				SEM (total) assigned value: 3.30 µg/kg				Total Nitrofurans Metabolites assigned value: 6.89 µg/kg			
	result, µg/kg	IS/% rec	RL	z-score	result	IS/% rec	RL	z-score	result	IS/% rec	RL	z-score
046	#				> 0.25	YES	0.25		#			
047	0.139	Yes/No	0.10		2.73	Yes/No	0.10	-0.8	5.87	Yes/No	0.10	-0.7
048	not detected				3.7	Y	0.5	0.5	#			
049	#				#				#			
050	#				3.79		0.25	0.7	#			
051	#				7.860	Y	0.8	6.3	#			
052	#				5.38	Y	0.5	2.9	#			
053	#				4.84	Y	0.5	2.1	9.33	Y	0.5	1.6
054	#				3.78	YES	0.13	0.7	#			

RL = reporting limit # = not analysed Y = internal standard used M = matrix-extracted calibration S = standard addition
z-scores outside $|z| > 2$ are shown in **bold**, see Section 5

Table 4: Additional Residues Reported

laboratory number	residue	result µg/kg	IS/% rec	RL
023	AMAZ (total)	1.11	118%	
030	AMAZ (total)	0.07	-	0.05

Table 5: Participants' Comments

laboratory number	comments
002	six point for Standard curve =0.0,0.1,0.25,0.5,1.0,2.0 (Åµg/kg),AHD r2=0.9992,AOZ r2=0.9997,SEM r2=0.9998, Based on Journal of Chromatography B.,691(1997) by LCMSMS.
003	Sample was tested with 5091AHD, 5091AOZ and SEM ELISA's for respectively AHD, AOZ and SEM.
010	SPK Recovery AOZ: 85.4% SPK Recovery AHD: 113.2% SPK Recovery SEM: 97% SPK Recovery AMOZ: 99.4%
012	Analyst: Hung, Cuc Method: 05.2/CL1/ST 03.69
019	LoQ used in place of CCbeta
023	We detect the fish sample with REAGEN ELISA test kits(AHD ELISA test kit , AMOZ ELISA test kit ,AOZ ELISA test kit, SEM ELISA test kit), Total Nitrofurans Metabolites is the sum of these four.
024	We report the LOQs of the nitrofurans metabolites.
028	we have received this sample in bad condition.
041	samples were analyzed on 08 November 2022
042	Method name used:Ministry of Agriculture Announcement No. 783 - 1-2006
043	Reporting Limit for AHD is 0.5ug/kg; Reporting limit for AMOZ is 0.25ug/kg. Reporting limit is the LOQ.
050	LOD AMOZ: 0.20 ug/kg
051	inadequate storage condition of sample received
053	Our laboratory works with matrix curve

comments are as submitted by participants but some may have been edited to maintain participant anonymity

Table 6: Assigned Values and Standard Deviations for Proficiency

analyte	data points, <i>n</i>	assigned value, x_a	units	uncertainty, <i>u</i>	standard deviation for proficiency, σ_p
AHD (bound)		not set			
AHD (total)	35	1.66	µg/kg	0.04	Horwitz [11] 0.366
AOZ (bound)		not set			
AOZ (total)	42	1.76	µg/kg	0.05	Horwitz [11] 0.387
SEM (bound)		not set			
SEM (total)	38	3.30	µg/kg	0.12	Horwitz [11] 0.727
Total Nitrofurans Metabolites	7	6.89	µg/kg	0.47	Horwitz [11] 1.52

Table 7: Number and Percentage of z-Scores where $|z| \leq 2$

analyte	number of scores where $ z \leq 2$	total number of scores	% $ z \leq 2$
AHD (bound)		na	
AHD (total)	34	44	77
AOZ (bound)		na	
AOZ (total)	44	50	88
SEM (bound)		na	
SEM (total)	34	46	74
Total Nitrofurans Metabolites	11	16	69

na = not applicable

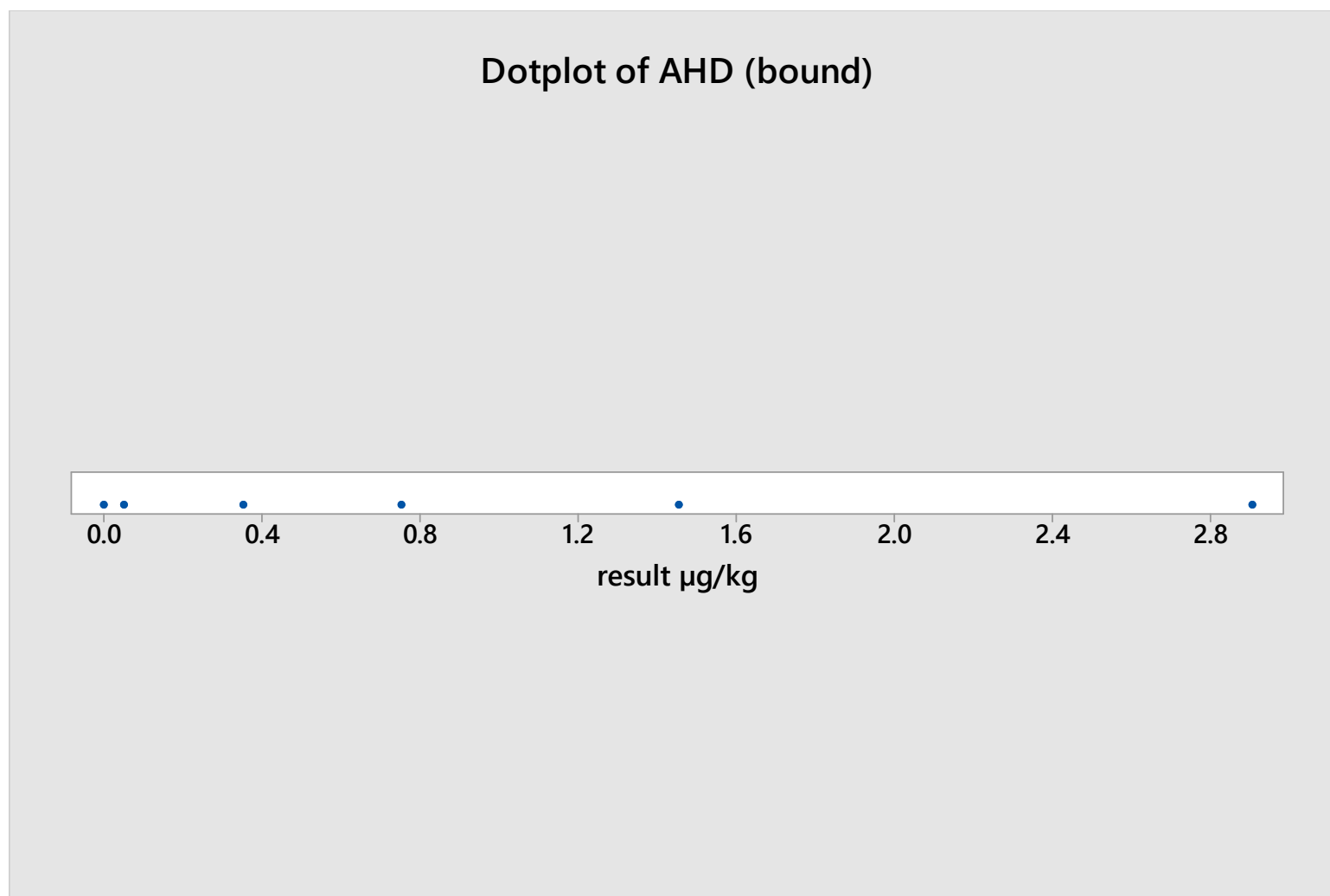


Figure 1: Results for AHD (bound)

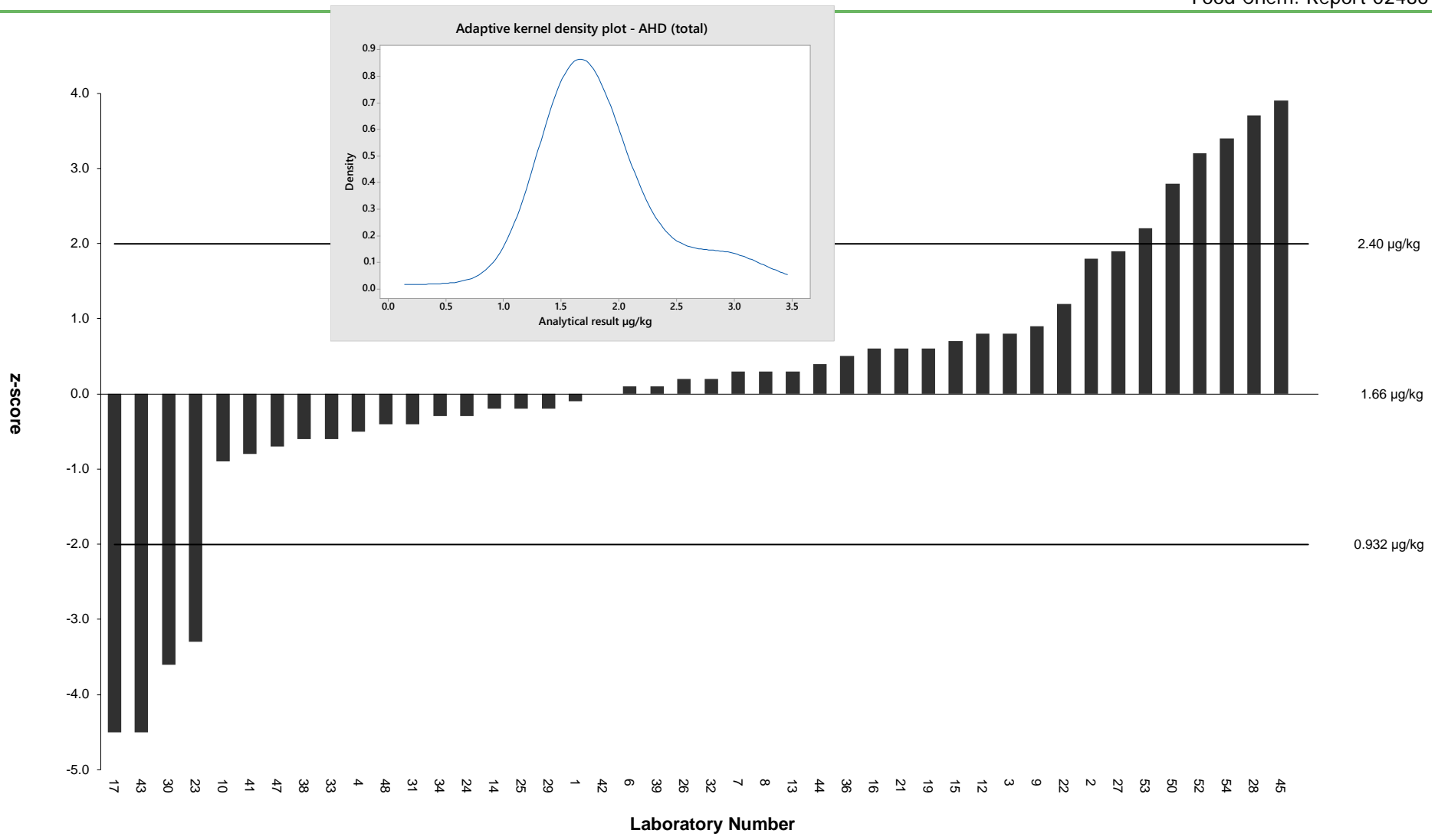


Figure 2: z-Scores for AHD (total)
 insert shows a plot of the distribution of results

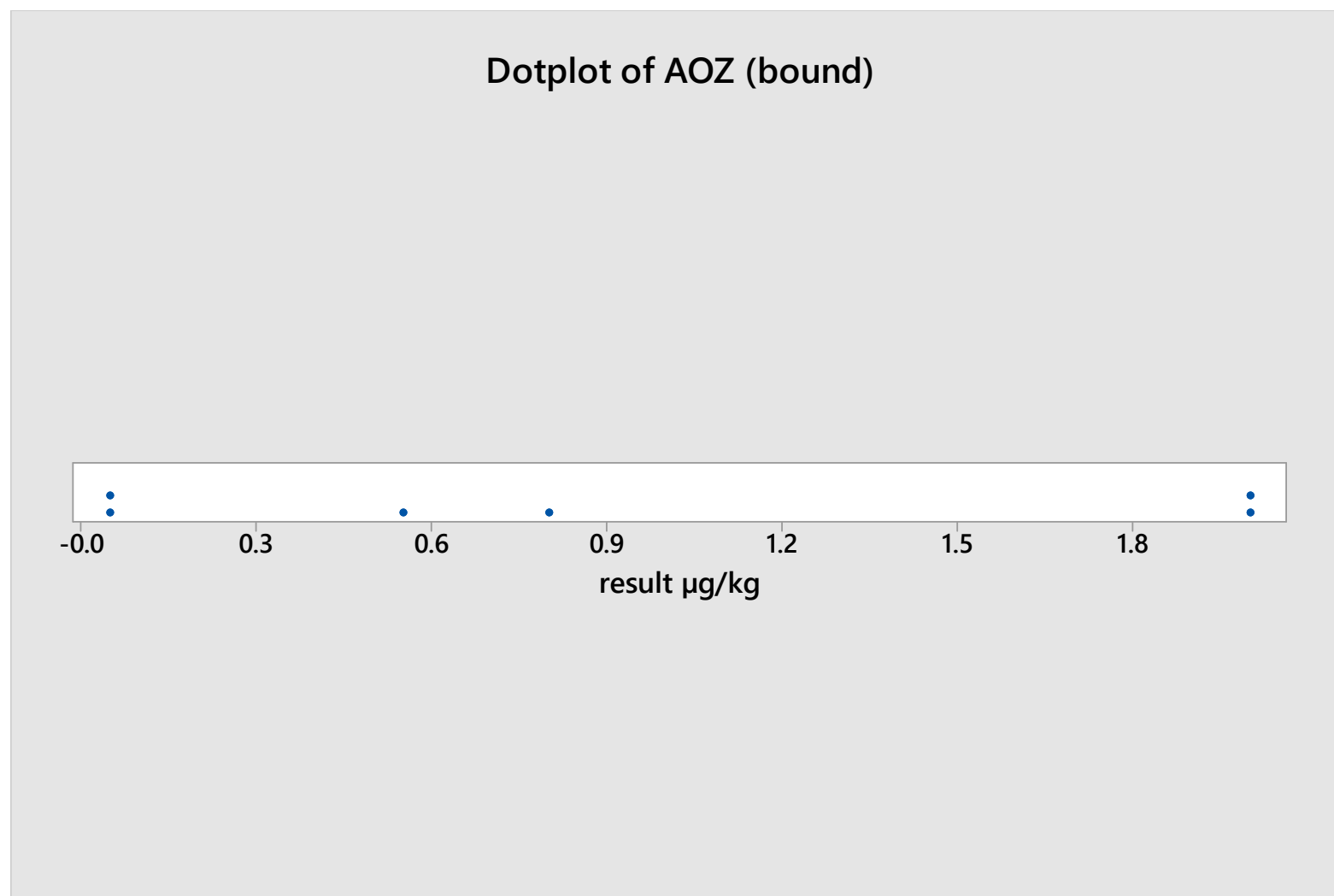


Figure 3: Results for AOZ (bound)

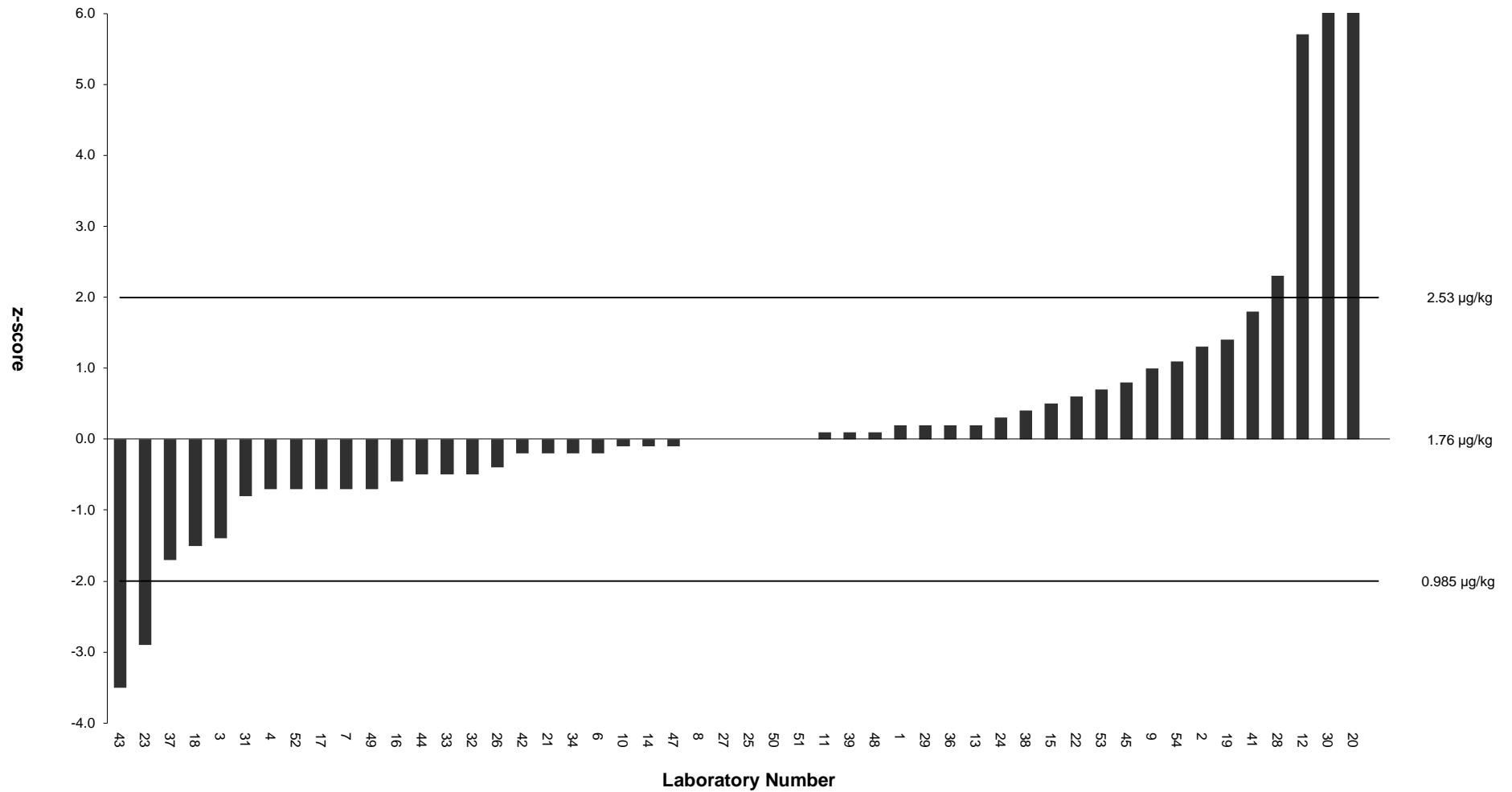


Figure 4: z-Scores for AOZ (total)

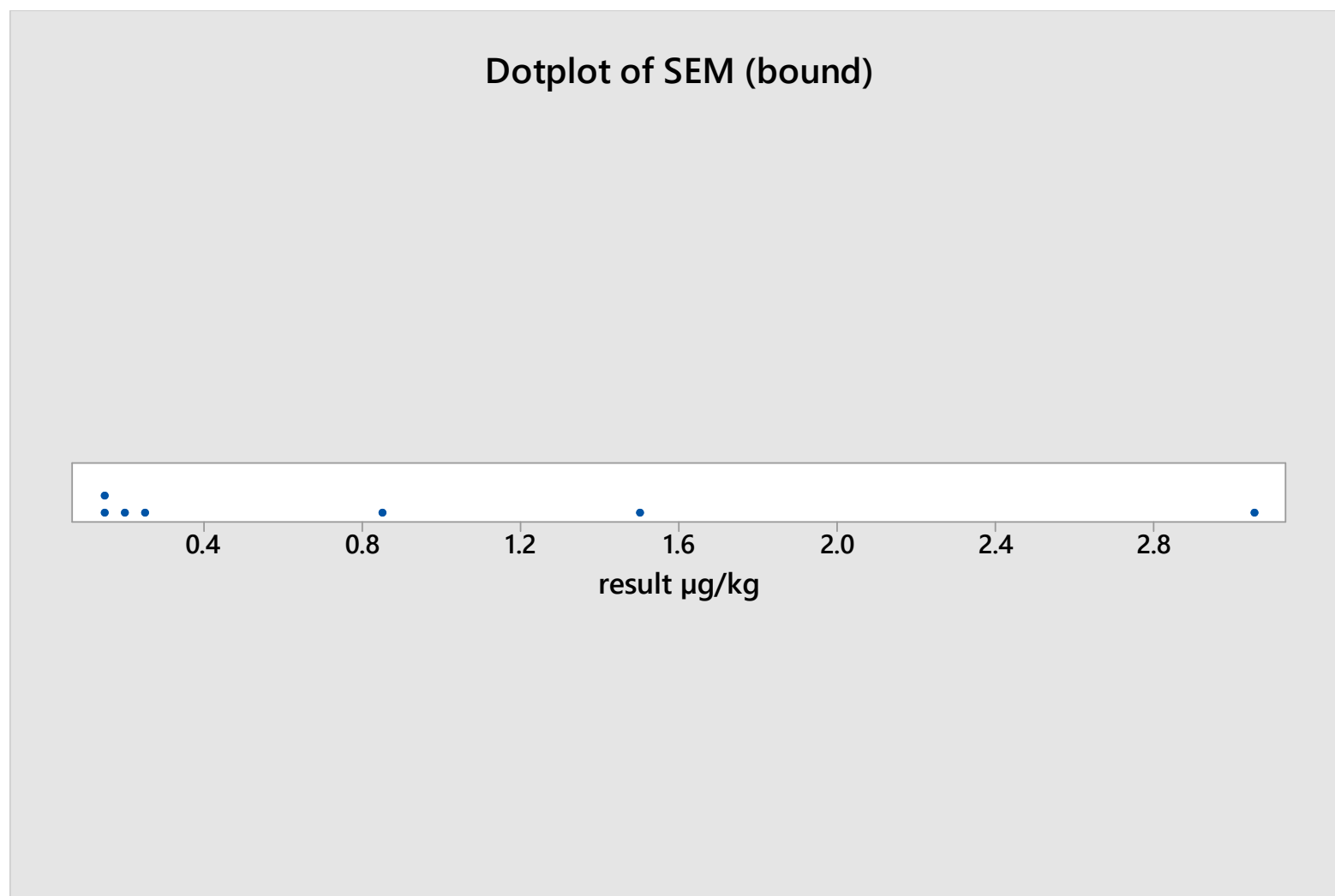


Figure 5: Results for SEM (bound)

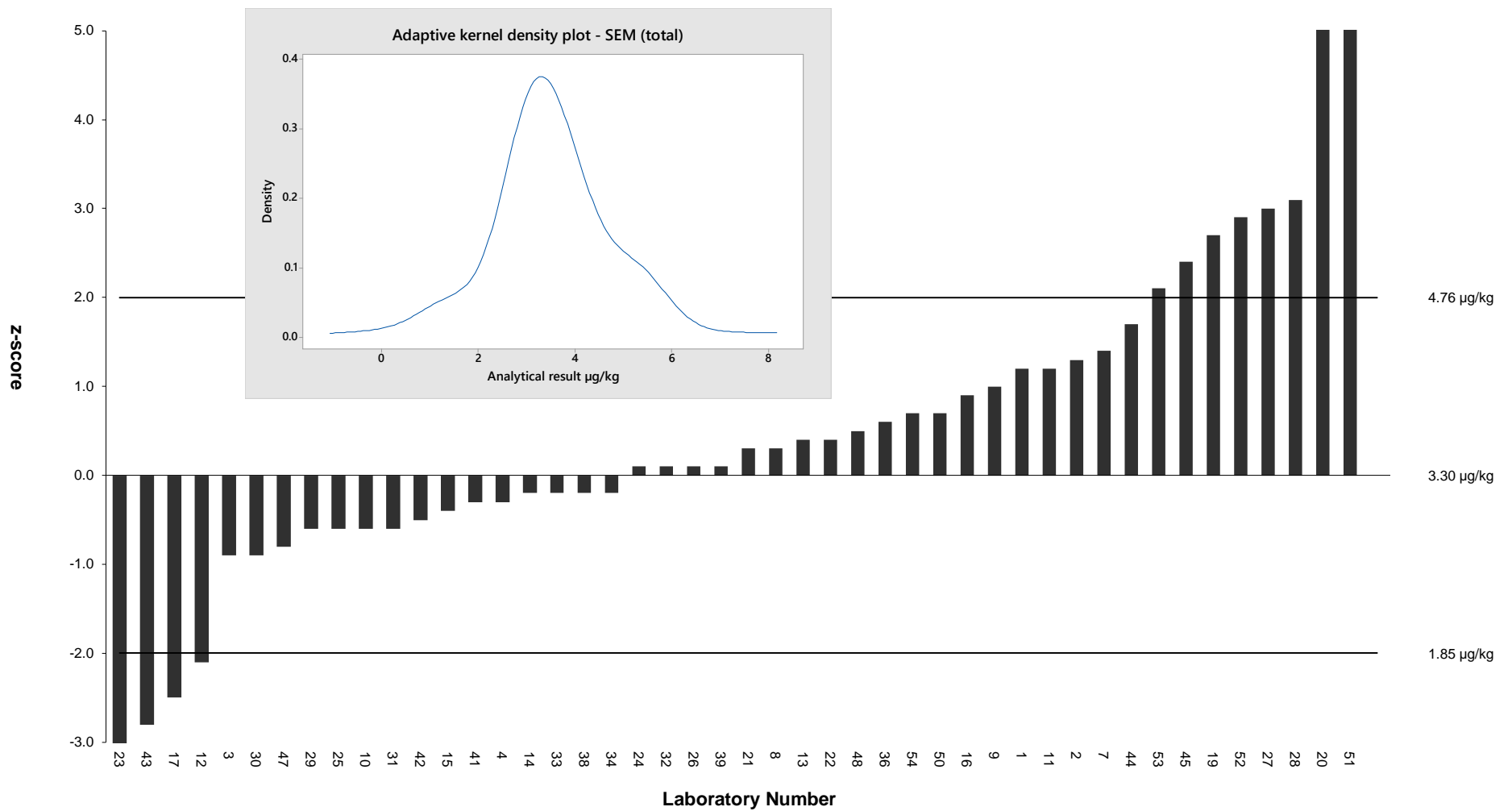


Figure 6: z-Scores for SEM (total)

insert shows a plot of the distribution of results

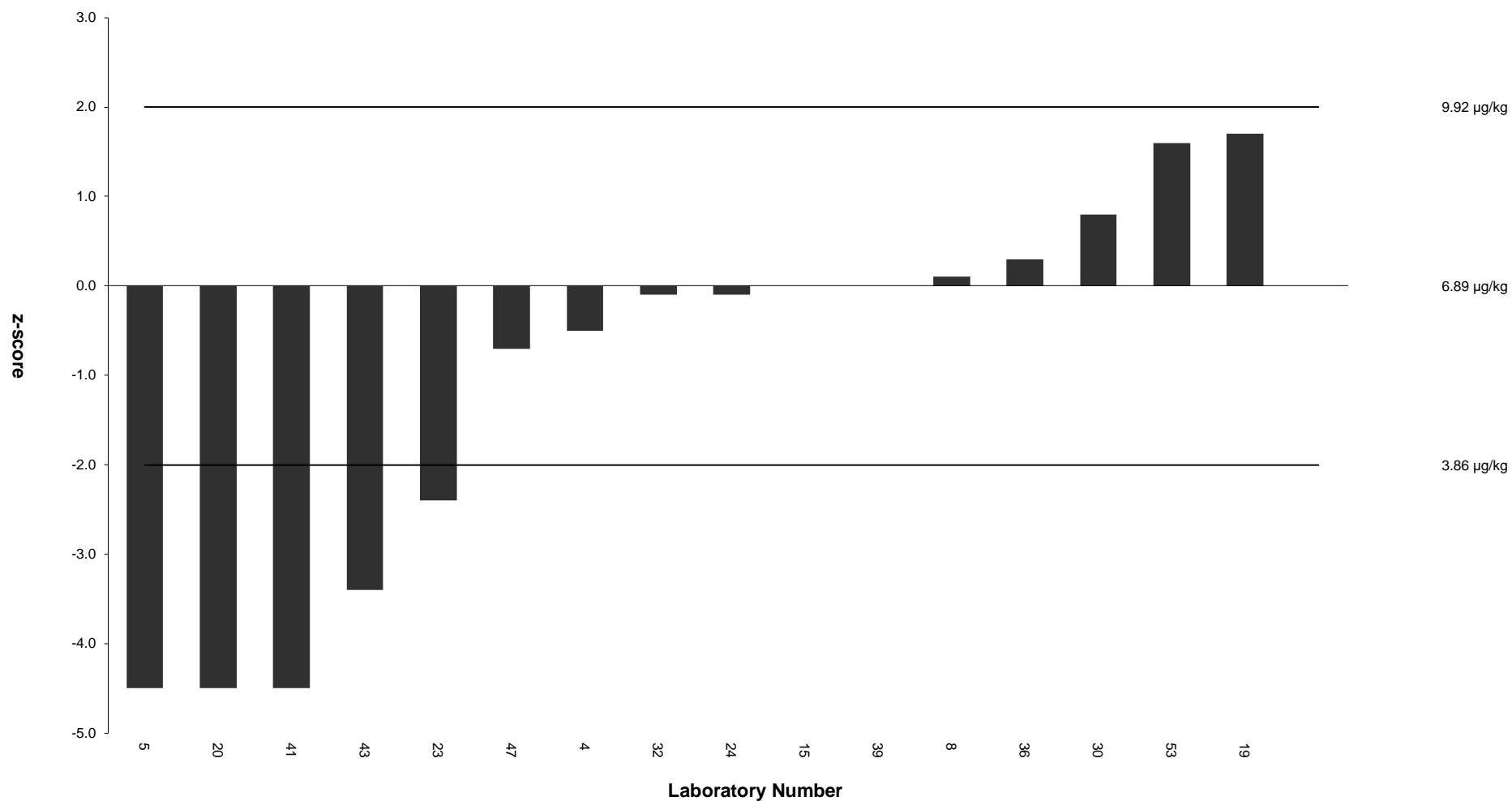


Figure 7: z-Scores for Total Nitrofuran Metabolites

APPENDIX I: Analytical Methods Used by Participants

Methods are tabulated according to the information supplied by participants, but some responses may have been combined or edited for clarity. Text that appears as unreadable symbols are derived from entries made using non-Western characters.

Is the Method Used Accredited?	laboratory number
no	003 004 016 023 025 030 036 044 051
yes	001 002 005 006 008 011 012 013 014 017 018 020 021 022 024 027 028 029 031 033 035 037 038 039 040 041 042 043 045 046 047 048 049 050 052 054

What is Your Method Based On?	laboratory number
International Standard	028 038 050
National Standard	005 006 011 020 021 022 024 025 029 033 042 045
Paper Published In An International Journal	004 014 017 036 037 043 054
Manufacturer/Kit Instructions/Technical Note	003 016 018 023 027 030 035 039 041
In house method	001 002 008 012 013 031 040 044 046 047 048 049 051 052

Sample Weight (g)	laboratory number
<1	003 018 027
≥1 - <2	002 004 008 011 013 016 020 023 028 030 031 033 035 039 040 041 043 045 046 047 048 050
≥2 - <5	005 012 014 017 021 022 024 025 029 036 037 042 044 051 052 054
≥5 - <10	001 006 038 049

Extraction Procedure	laboratory number
cold solvent extraction at atmospheric pressure	003 017 029 038 039 040 044 045 046 048 049 051
hot solvent extraction at atmospheric pressure (e.g. soxhlet)	023
maceration/homogenisation	006 014 027 035 052
shaking	001 008 018 020 033 043 047 050
sonicate/ultrasonic bath	028 031
vortex mix	004 005 011 012 013 016 022 024 025 030 037 041 042 054
acid hydrolysis using incubator shaker	036

Extraction Solvent Components	laboratory number
ethyl acetate	002 003 005 008 012 013 016 017 018 020 022 023 024 025 028 029 031 033 035 039 040 041 042 043 045 046 047 048 050 051 054
hexane	004 035 051
methanol	028 037 044
phosphate buffer	014
water	011 028 052
0.125M HCl	036
0.1mol/L hydrochloric acid	038
CLORHIDRIC ACID	001
HCl	006
TMPD	027

Extraction Time (mins)	laboratory number
≥0.1 - <1	005 006 049
≥1 - <2	024 027 029 039
≥2 - <5	022 023 031 037 050 052
≥5 - <10	013 035 038 043
≥10 - <30	004 018 020 025 030 033 040 048 054
≥30 - <60	002 012 017 041 044 046 047
≥60	001 008 011 014 045 051

Enzyme Deconjugation Used?	laboratory number
no	001 004 005 006 011 012 014 016 017 018 020 021 022 023 024 025 027 030 031 036 037 039 040 043 044 045 046 047 048 049 051 052 054
yes	008 013 028 033 038 041

Enzyme Used	laboratory number
beta-glucuronidase	013 033 041
2-NBA	008
2-Nitrobenzaldehyde	040
NBA/DMSO	038
no	043 045
overnight hydrolysis and simultaneous derivatization	004

Sample Work Up	laboratory number
Acid Hydrolysis with HCl	001 004 005 013 017 018 020 022 023 024 028 036 042 043 046 048 052
centrifuge	008 011 027 030 033 035 037 040 044 051
defatted with hexane	012 031 039
evaporate	016 041
filter	006
pH adjustment	014 025 029 038 047
centrifuge and defatted with hexane	045

Sample Clean-up Technique	laboratory number
filter	044 048
liquid/liquid extraction	001 004 005 012 018 020 022 024 025 029 031 033 037 043 045 046 047 048 049 051 054
solid phase extraction (SPE) (column/cartridge)	004 006 011 014 027 036 038 052
solid phase extraction (SPE) (dispersive)	008
solvent exchange	023 042
none	013 017 040 041

SPE Sorbent Type	laboratory number
C18	014
MCX	027
Oasis HLB	004 011 036
Chem Elut	038
chem elute	006
No	043
no sorbent used	045
none	047
SDB-L	052
Strata SDB-L	008

Calibrations	laboratory number
solvent	011 018 025 031 044 048
matrix-matched	001 004 013 014 017 020 021 022 024 028 029 037 040 043 045 046 049 050 052 054
single-level	046
multi-level	004 006 008 013 018 027 028 038 041 042 044 047 048
standard addition	005 008 012 023 033 035 036 047 051

Type of Internal Standard Added	laboratory number
none	023 024 038 041 044
Stable Isotope Labelled Analogue	004 005 006 008 011 012 013 014 017 020 022 025 027 028 029 031 033 036 037 042 043 045 046 048 049 052
structural analogue	001 040 051 054
AOZ-D4 and AMOZ-D5	047

Method of Separation	laboratory number
ELISA	003 016 018 023 030 035 039 041
HPLC	001 002 004 005 006 008 011 012 013 014 017 020 022 024 025 028 029 031 033 036 042 043 044 045 046 047 048 049 052
LCMS/MS	040
LCMSMS	027 037 038
UPLC MSMS	054
UPLC-MS-MS	051

HPLC Column Packing	laboratory number
C18	001 002 004 005 006 011 012 013 014 017 020 022 024 025 027 028 029 031 033 036 038 040 042 043 044 045 046 047 048 049 050 052
C8	037 054
Phenyl	008

Mobile Phase Components	laboratory number
ammonium acetate	001 002 012 014 020 022 024 028 029 036 038 042 045 047 048 050
ammonium formate	004 017 031 043 044 046 052
ethanoic acid (acetic acid)	006
acetonitrile	001 006 013 022 025 027 033 040 044 046 051 052
formic acid (methanoic acid)	027 037 044 049
methanol	004 005 008 011 020 022 024 028 029 044 045 047 048 049 052
water	008 028 029 040 044 045 048 051
Acetic Acid	040
formic acid in acetonitril	012

HPLC Post Column Derivatisation	laboratory number
none	004 005 006 008 011 012 013 014 020 024 025 027 028 029 031 033 036 038 040 042 043 045 046 047 048 049 054

HPLC Detector Type	laboratory number
MS-MS	001 002 004 005 006 008 011 012 013 014 017 020 022 024 025 027 028 029 031 033 036 037 038 040 042 043 044 045 046 047 048 050 052 054

ELISA Test Kit Name	laboratory number
-	040
5091AHD, 5091AOZ and 5091SEM	003
AOZ AMOZ SEM AHD ELISA KIT	030
maxsignal perkin elmer	041
No	038 045
REAGEN AOZ ELISA TEST KIT ,REAGEN AMOZ 023 ELISA TEST KIT ,REAGEN SEM ELISA TEST KIT,REAGEN AHD ELISA TEST KIT	
RIDASCREEN	018
RIDASCREEN Nitrofurane (AMOZ) and RIDASCREEN Nitrofurane (AOZ)	035
RIDASCREEN Nitrofurane R 3724 SEM; R 3703 AOZ; R3722 AMOZ; R 3713 AHD	016
TABP Nitrofurane ELISA Diagnostic Kit	039

ELISA Kit Manufacturer	laboratory number
R-Biopharm	003 016 018 035
AOZ SEM AHD MAX SIGNAL PERKIN ELMER INC & AMOZ TAIWAN ADVANCE BIO- PHARMACEUTICAL INC	030
none	045
perkin elmer	041
REAGEN	023
TABP	039

ELISA Antibody Description	laboratory number
monoclonal	041
polyclonal	003 023 039
no info	018 030 035 040 045

ELISA Standard Material	laboratory number
other (please specify)	040 045
provided by test manufacturer	003 016 018 023 030 035 039 041

ELISA Number of Standards	laboratory number
6	003 016 018 023 030 035 039 041
-	040
0	045

ELISA Time Requirement for Testing (min) [not sample preparation]	laboratory number
≥30 - <60	003 023 030 035 039 041
≥60 - <90	016 018

ELISA Calculation of Results	laboratory number
4 parameter	003
cubic spline	016 018 035
logit / log	023 030 039 041
-	040

ELISA Extraction Solvent	laboratory number
-	040
ethyl acetate	023 039
ethyle acetate	041
n-Hexane	030

ELISA Sample Extraction (weight/volume, g/ml)	laboratory number
<0.5	018 039
≥0.5 - <1	035
≥1 - <2	003 016 023 030 041

ELISA Dilution Factor of Sample Preparation	laboratory number
1:2	016 018 023 030 035 039 041

AHD (bound)

Limit of Detection ($\mu\text{g}/\text{kg}$)	laboratory number
$\geq 0.01 - < 0.1$	002
$\geq 0.1 - < 1$	005 040

CC alpha (decision limit) ($\mu\text{g}/\text{kg}$)	laboratory number
$\geq 0.1 - < 1$	040

CC beta (detection capability) ($\mu\text{g}/\text{kg}$)	laboratory number
$\geq 0.1 - < 1$	040

MS-MS Transitions Monitored	laboratory number
249>178,134,104	040

For MS only, Single Ions Monitored, m/z	laboratory number
-	040

For HR -MS only, Ions Monitored, m/z	laboratory number
-	040

Wavelength (absorbance)(nm)	laboratory number
-	040

Wavelength (excitation)(nm)	laboratory number
-	040

Wavelength (emission)(nm)	laboratory number
-	040

AHD (total)

Limit of Detection ($\mu\text{g}/\text{kg}$)	laboratory number
≥ 0.01 - < 0.1	002 023 039 047
≥ 0.1 - < 1	001 010 012 014 016 021 027 031 033 036 042 045 048 052 054
≥ 1 - < 10	006 013 029

CC alpha (decision limit) ($\mu\text{g}/\text{kg}$)	laboratory number
≥ 0.01 - < 0.1	023
≥ 0.1 - < 1	001 012 013 033 047 048 052 054
≥ 1 - < 10	014 029

CC beta (detection capability) ($\mu\text{g}/\text{kg}$)	laboratory number
≥ 0.1 - < 1	001 012 014 033 046 047 048 052 054
≥ 1 - < 10	023 029

MS-MS Transitions Monitored	laboratory number
249 > 104, 249 > 134, 249 > 178	029
249,1 -- 134/104	012
249.03 > 133.93 and 249.03 > 178.30	013
249.088 > 104.071, 249.088 > 133.917, 249.088 > 178.125	031
249.09 > 134.07	054
249.1 > 104 - 249.1 > 134	046
249.1 > 134 , 249.1 > 104	048
249.1 > 134.0	045
249.1 > 134.1	033
249.1 > 134.2, 249.1 > 104.2	014
249.11 > 133.93, 249.11 > 103.85	047

MS-MS Transitions Monitored (continued) **laboratory number**

249.15 >133.98	027
249/104, 249/134	010
249>134	006
249>134, 249>178, 249>104	001
249>134,249>104	052
AHD:249.2>134.1	042

Wavelength (absorbance)(nm) **laboratory number**

450	023
450 nm	016
450/650	039

AOZ (bound)

Limit of Detection ($\mu\text{g}/\text{kg}$) **laboratory number**

≥ 0.01 - < 0.1	002
≥ 0.1 - < 1	005 040

CC alpha (decision limit) ($\mu\text{g}/\text{kg}$) **laboratory number**

≥ 0.1 - < 1	040
--------------------	-----

CC beta (detection capability) ($\mu\text{g}/\text{kg}$) **laboratory number**

≥ 0.1 - < 1	040
--------------------	-----

MS-MS Transitions Monitored **laboratory number**

236>134,104	040
-------------	-----

For MS only, Single Ions Monitored, m/z **laboratory number**

-	040
---	-----

For HR -MS only, Ions Monitored, m/z	laboratory number
-	040

Wavelength (absorbance)(nm)	laboratory number
-	040

Wavelength (excitation)(nm)	laboratory number
-	040

Wavelength (emission)(nm)	laboratory number
-	040

AOZ (total)

Limit of Detection ($\mu\text{g}/\text{kg}$)	laboratory number
≥ 0.01 - < 0.1	002 010 016 018 023 039 047
≥ 0.1 - < 1	001 011 012 014 017 021 027 031 033 036 037 042 043 045 048 051 052 054
≥ 1 - < 10	006 013 020 029

CC alpha (decision limit) ($\mu\text{g}/\text{kg}$)	laboratory number
≥ 0.01 - < 0.1	012 023
≥ 0.1 - < 1	001 011 013 014 033 043 047 048 052 054
≥ 1 - < 10	029

CC beta (detection capability) ($\mu\text{g}/\text{kg}$)	laboratory number
≥ 0.1 - < 1	001 011 012 014 018 033 043 046 047 048 052 054
≥ 1 - < 10	023 029

MS-MS Transitions Monitored	laboratory number
236 > 134, 236 > 149, 236 > 104	029
236 > 134.2	037
236,1-104/134	012
236.0>134.2, 236.0>104.2	014
236.03>103.86 and 236.03>133.87	013
236.05 >133.98	027
236.08 > 133.93,236.08 > 103.85	047
236.0912>104.03	054
236.1 > 104 - 236.1 > 134	046
236.1 > 134 , 236.1 > 104	048
236.1>133.9	045
236/134,236/104,236/78	010
236>104.071, 236>133.917, 236>149	031
236>133.9; 236>104	043
236>134	006 033
236>134, 236>104	001
236>134, 236>104	052
AOZ 236>104.1 AOZ 236>134.1	051
AOZ:236>134	042

Wavelength (absorbance)(nm)	laboratory number
450	018 023
450 nm	016
450/650	039

SEM (bound)

Limit of Detection ($\mu\text{g}/\text{kg}$)	laboratory number
≥ 0.01 - < 0.1	002 047
≥ 0.1 - < 1	005

CC alpha (decision limit) ($\mu\text{g}/\text{kg}$)	laboratory number
≥ 0.1 - < 1	047

CC beta (detection capability) (µg/kg)	laboratory number
≥0.1 - <1	047

MS-MS Transitions Monitored	laboratory number
209.05 > 166.00, 209.05 > 192.03	047

SEM (total)

Limit of Detection (µg/kg)	laboratory number
≥0.01 - <0.1	002 010 023 039 047 051
≥0.1 - <1	001 012 014 016 017 021 027 031 033 036 042 043 045 048 052 054
≥1 - <10	013 020 029

CC alpha (decision limit) (µg/kg)	laboratory number
≥0.01 - <0.1	013 023
≥0.1 - <1	001 012 033 043 047 048 052 054
≥1 - <10	014 029

CC beta (detection capability) (µg/kg)	laboratory number
≥0.1 - <1	001 012 014 033 043 046 047 048 052 054
≥1 - <10	023 029

MS-MS Transitions Monitored	laboratory number
208.98 > 192.08	027
209 > 192, 209 > 166, 209 > 134	029
209,1-192,1/166,1	012
209.01 > 166.05	054
209.03/166, 209/192	010
209.03 > 77.81 / 209.03 > 165.50 / 209.03 > 166.0 / 209.03 > 192.2	013
209.05 > 166.00, 209.05 > 192.03	047
209.1 > 166.0	045

MS-MS Transitions Monitored (continued)	laboratory number
209.1 > 166.1 , 209.1 > 192.1	048
209.1 > 192.1 - 209.1 > 166.1	046
209.1>166.1	033
209.1>166.1 209.1>192.1	051
209.1>192.3, 209.1>166.3	014
209.125>133.97, 209.125>166.196, 209.125>192.125	031
209>166, 209>192	001
209>166.1, 209>192.1	052
209>191.7; 209>166.1	043
SEM:209>166	042

Wavelength (absorbance)(nm)	laboratory number
450	023
450 nm	016
450/650	039

Total Nitrofuran Metabolites

Limit of Detection (µg/kg)	laboratory number
≥0.01 - <0.1	023 039 047
≥0.1 - <1	036 043

CC alpha (decision limit) (µg/kg)	laboratory number
≥0.01 - <0.1	023
≥0.1 - <1	043 047

CC beta (detection capability) (µg/kg)	laboratory number
≥0.1 - <1	043 047
≥1 - <10	023

MS-MS Transitions Monitored**laboratory number**

AHD = 249.11 > 133.93, 249.11 > 103.85, AOZ = 047
236.08 > 133.93, 236.08 > 103.85 and SEM =
209.05 > 166.00, 209.05 > 192.03

Wavelength (absorbance)(nm)**laboratory number**

450	023
450/650	039

APPENDIX II: Fapas[®] SecureWeb, Protocol and Contact Details

1. Fapas[®] SECUREWEB

Access to the secure area of our website is only available to participants in our proficiency tests. Please contact us if you require a UserID and Password. Fapas[®] SecureWeb allows participants to:

- Obtain their laboratory numbers for the proficiency tests in which they have participated.
- View the results they submitted in past and current proficiency tests.
- Submit their results and methods for current tests.
- Review future tests they have ordered.
- Order proficiency tests, reference materials and quality control materials.
- Freely download copies of reports (PDF file), of proficiency tests in which they have participated.
- View charts of their z-scores obtained in previous Fapas[®] – Food Chemistry proficiency tests.

2. PROTOCOL

The Protocols [7, 8] set out how Fapas[®] – Food Chemistry is organised. Copies can be downloaded from our website.

3. CONTACT DETAILS

This report was prepared and authorised on behalf of Fapas[®] by Claire Williamson (Round Coordinator). Participants with any comments or concerns about this proficiency test should contact:

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