Asia Pacific Laboratory Accreditation Cooperation

APLAC T050

Prawn Nitrofuran Metabolites Proficiency Testing Program

FINAL REPORT

China National Accreditation Service for Conformity Assessment (CNAS)

November 2008

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Contents

APLAC T050

Prawn Nitrofuran Metabolites Proficiency Testing Program

FINAL REPORT

1. Instruction

This report summarizes the results of APLAC Prawn Nitrofuran Metabolites Proficiency Testing Program T050.

This program was conducted in accordance with the interlaboratory testing scheme specified in *ISO/IEC Guide 43-1*, covered quantitative tests on nitrofuran metabolite contents in prawn sauce samples. This program applies only to the use of interlaboratory comparisons of the purpose of proficiency testing. The aim of this program is to assess the performance of individual laboratories for specific tests. It does not include determining the effectiveness and precision of testing methods.

2. Main Relevant Parties

Asia Pacific Laboratory Accreditation Cooperation (APLAC) approved the program.

2.1 Organizing and Coordinating Bodies

APLAC T050 Program was organized by China National Accreditation Service for Conformity Assessment (CNAS) and coordinated by Technical Centre of Liaoning Entry-Exit Inspection and Quarantine Bureau of People's Republic of China.

2.2 Summary of Participants and Their Accreditation Situations

The members of APLAC, EA, IAAC and some other laboratory accreditation bodies were invited to nominate laboratories to participate in this program. A total of 39 laboratories from 19 economies were nominated.

2 laboratories could not report any results till the testing deadline, 37 laboratories reported the testing results of AOZ, in which 28 laboratories were accredited for testing AOZ; 36 laboratories reported the testing results of AMOZ, in which 27 laboratories were accredited for testing AMOZ; 31 laboratories reported the testing results of AHD and SEM, in which 23 laboratories were accredited for testing AHD and SEM by their accreditation bodies.

Each participating laboratory was endowed with an exclusive lab code. All references to the laboratories associated with this program are with these codes, respectively. The numbers of laboratories with respect to their accreditation bodies are listed in Table 1.

Table 1 Summary of Participants

Economy	Accreditation Body	Number of Labs
Argentina	Organismo Argentino de Acreditación (OAA)	2
Australia	National Association of Testing Authorities (NATA)	3
Brasil	General Accreditation Coordination – Cgcre/Inmetro	2
Bulgaria	Bulgarian Accreditation Service	1
Canada	Standard Council Of Canada (SCC)	4
Chile	Instituto Nacional de Normalización (INN)	1
China	China National Accreditation Service for Conformity Assessment (CNAS)	4
France	COFRAC	1
Germany	Deutsche Akkreditierungsstelle Chemic GmbH	1
Indonesia	National Accreditation Body of Indonesia (KAN)	4
Israel	Israel Accreditation (ISRAC)	1
Japan	The Japan Accreditation Board for Conformity Assessment (JAB)	2
Malaysia	Department of Standards Malaysia (Standards Malaysia)	3
New Zealand	International Accreditation New Zealand (IANZ)	1
Norway	Norwegian Accreditation (NA)	2
Poland	Polish Centre for Accreditation	2
Portugal	IPAC- Instituto Português de Acreditação, I.P.	1
Romania	RENAR-Romanian Accreditation Association	3
Switzerland	Swiss Accreditation Service (SAS)	1

3. Statistical Design

3.1 Agreement of Testing Methods

Nitrofuran Drugs were used in aquiculture, the metabolites of these drugs come from some aquatic and marine products could stay in human bodies for relative long time, and cause human diseases, such as queasiness, retch, diarrhea and haemolytic anemia. These metabolites should be monitored to protect human health.

There are various methods for quantifying nitrofuran metabolite residues in animal tissues, however, there was no evidence on harmonization of these methods. Prior to this program, a series of experiments were carried out in our laboratory using some typical methods for prawn. It indicated that extraction and purification efficiency are main factors leading bias to the testing results. Thus, an operationally-defined pretreatment protocol was suggested in the Instruction to Participants in this program.

3.2 Three Samples Employed

The accuracy of testing results may be different on samples with different residue levels. Consequently, discrepancy probably occurs for samples with different residue levels tested in different laboratories.

In this program, three samples with increasing contents of nitrofuran metabolites were employed, in which three levels were designed. The lowest level was set at restriction level, the highest level was about 3 times the lowest level, one level inserted between the highest and lowest levels. The accuracy of testing results at these different levels will be examined in this proficiency testing program.

3.3 Methods for Statistical Assessments

When the agreement of the testing results were good enough, the participants' performance should be assessed using z-scores calculated by robust statistical method, **median** and **normalized interquartile range (NIQR)** intended to be used as the statistics for z-score calculation, as shown in Appendix D.

Individual **z-score** for each result was used as the assessment for the ability of an individual participating laboratory. Statistical graphs, such as **histograms** and **ordered z-score bar charts**, were regarded as references to the overall effectiveness of this program.

3.4 Measurement Uncertainty

To ensure the **traceability**, the measurement uncertainty of the assigned values were evaluated and limited in sample preparation. It enables the assessments more reasonable.

4. Description of Samples

4.1 Preparation and Distribution

Each **gross sample** was prepared by adding designed amounts of nitrofuran metabolites to the blank fresh prawn sauce.

Respectively, each gross sample was divided and packed into aseptic bags to make into testing samples.

The testing samples were randomly labeled and recorded. Testing samples packed with the Instructions for Participants (see Appendix E) were distributed directly to participating laboratories as requested.

Note: *For the convenience of description in this report, these three gross samples are denoted as Gross Sample A, Gross Sample B and Gross Sample C, the testing samples prepared from each corresponding gross sample are denoted as Sample A, Sample B and Sample C, and the results obtained on these samples are denoted as Result A, Result B and Result C, respectively.*

4.2 Homogeneity and Stability

The samples randomly selected from each gross sample were determined for their homogeneity and stability. Details of sample homogeneity and stability check are described in Appendix C.

5. Testing Results and Assessments

5.1 Methods for Assessments

For an individual participating laboratory, z-scores were employed to assess the proficiency on specific testing. The methods for calculating z-scores are outlined in Appendix D. As a general rule, according to *ISO/IEC GUIDE 43-1*, any absolute value of z-score falls in the range of 2 to 3 is regarded as a questionable result, while any absolute value of z-score exceeds 3 is regarded as an unsatisfactory result.

The participants are encouraged to review, if any, the questionable or unsatisfactory results. It is suggested that the participants refer to the main technical aspects given in clause 6 of this report.

5.2 Nitrofuran Metabolite AOZ

37 participating laboratories reported testing results of AOZ contents in samples of A, B and C, the results are listed together with the testing methods in Appendix A. The **standard uncertainties** of the assigned values were estimated. The statistics are listed in Table 2.

Table 2 Statistics for Assessments on Testing Results of AOZ in Samples of A, B and C

Gross Sample	Assigned Value (μg/kg)	NIQR (μg/kg)	Standard Uncertainty of Assigned Value (μg/kg)
Gross Sample A	4.3	1.4	0.6
Gross Sample B	7.5	2.4	1.0
Gross Sample C	11.7	5.2	2.1

4(10.8%) laboratories made questionable results about sample A. 2 (5.4%) laboratories made questionable results about sample B. 3 (8.1%) laboratories made questionable results about sample C. The lab codes with questionable results are listed in Table 3.

Table 3 Laboratories Made Questionable Results of AOZ

z-scores	Questionable ($2 \leq \|z\| < 3$)
z-score of sample A (z_A)	T050-023,T050-025,T050-032, T050-033
z-score of sample B (z_B)	T050-025, T050-033
z-score of sample C (z_C)	T050-033, T050-034, T050-035

5.3 Nitrofuran Metabolite AMOZ

36 participating laboratories reported testing results of AMOZ contents in samples of A, B and C, the results are listed together with the testing methods in Appendix A. The standard uncertainties of the assigned values were estimated. The statistics are listed in Table 4.

Table 4 Statistics for Assessment on Testing Results of AMOZ in Samples of A, B and C

Gross Sample	Assigned Value (μg/kg)	NIQR (μg/kg)	Standard Uncertainty of Assigned Value (μg/kg)
Gross Sample A	4.4	1.6	0.7
Gross Sample B	7.9	2.7	1.1
Gross Sample C	11.5	4.7	2.0

7(19.4%) laboratories made questionable results about sample A. 5 (13.9%) laboratories made questionable results about sample B. 4 (10.8%) laboratories made questionable or unsatisfactory results, in which 2(5.6%) laboratories reported unsatisfactory results about sample C. The lab codes with questionable results are listed in Table 5.

Table 5 Laboratories Made Questionable or Unsatisfactory Results of AMOZ

z-scores	Questionable ($2\leq\|z\|<3$)	Unsatisfactory ($\|z\|\geq3$)
z-score of sample A (z_A)	T050-007, T050-012, T050-015, T050-032, T050-033, T050-035, T050-037	----------
z-score of sample B (z_B)	T050-015, T050-028, T050-032, T050-033, T050-034	----------
z-score of sample C (z_C)	T050-033, T050-034	T050-015, T050-035

5.4 Nitrofuran Metabolite of AHD

31 participating laboratories reported testing results of AHD contents in samples of A, B and C, the results are listed together with the testing methods in Appendix A. The standard uncertainties of the assigned values were estimated. The statistics are listed in Table 6.

Table 6 Statistics for Assessment on Testing Results of AHD in Samples of A, B and C

Gross Sample	Assigned Value (μg/kg)	NIQR (μg/kg)	Standard Uncertainty of Assigned Value (μg/kg)
Gross Sample A	4.1	1.4	0.6
Gross Sample B	7.2	2.4	1.1
Gross Sample C	11.0	3.6	1.6

3(9.7%) laboratories made questionable results about sample A. 2 (6.5%) laboratories made questionable results about sample B. 5 (16.1%) laboratories made questionable results about sample C. The lab codes with questionable results are listed in Table 7.

Table 7 Laboratories Made Questionable Results of AHD

z-scores	Questionable ($2\leq\|z\|<3$)
z-score of Sample A (z_A)	T050-017, T050-033, T050-034
z-score of Sample B (z_B)	T050-019, T050-033
z-score of Sample C (z_C)	T050-006,T050-019, T050-029, T050-033, T050-034

5.5 Nitrofuran Metabolite of SEM

31 participating laboratories reported testing results of SEM contents in samples of A, B and C, the results are listed together with the testing methods in Appendix A. The standard uncertainties of the assigned values were estimated. The statistics are listed in Table 8.

Table 8 Statistics for Assessment on Testing Results of SEM in Samples of A, B and C

Gross Sample	Assigned Value (μg/kg)	NIQR(μg/kg)	Standard Uncertainty of Assigned Value (μg/kg)
Gross Sample A	5.0	2.2	1.0
Gross Sample B	7.6	3.4	1.5
Gross Sample C	12.5	4.3	1.9

1(3.2%) laboratories made unsatisfactory results about sample A. 2 (6.5%) laboratories made questionable results about sample B. 4 (12.9%) laboratories made questionable results about sample C. The lab codes with questionable results are listed in Table 9.

Table 9 Laboratories Made Questionable or Unsatisfactory Results of SEM

z-scores	Questionable ($2\leq\|z\|<3$)	Unsatisfactory ($\|z\|\geq3$)
z-score of Sample A (z_A)	---------	T050-024
z-score of Sample B (z_B)	T050-005, T050-024	
z-score of Sample C (z_C)	T050-013, T050-019, T050-033, T050-034	

6. Technical Commentary

In order to facilitate the participants to find the reasons of unsatisfactory results and improve their testing performance, main factors that influence the analytical results of nitrofuran metabolites contents are described as following.

- Extracting method employed;
- Cleaning-up method employed;
- Correcting results by recovery;
- Testing conditions;
- Using and storage of reference materials;
- Storage conditions of samples.

In this program, acidic solvents or polar organic solvents were used by some participants to extract nitrofuran metabolites from testing samples, it may lead to lower extraction efficiency than using alkaline solvents. Lower results were reported consequently. Extracting operation, such as oscillation, centrifugation and homogeneization, could cause loss of analytes if it is incomplete.

SPE methods were used by some participants for cleaning-up, however, unsuitable sort or volume of the elution solvents, or the unsuitable eluting rate may cause incomplete elution or loss of analyte. Some participants might not implement any cleaning-up steps, inaccurate results were obtained due to interference.

Some participants did not use reference materials properly. Different kind of internal standards

may lead to different response on the same equipment, it should be corrected, unfortunately, these participants could not pay attention to the discrepancy.

According to the Invitation, Instruction to Participants of this program, the LODs should be better than 0.2μg/kg, some participants could not match the condition, their results on the sample with lowest level are out of control.

According to some participants' feadback for the Interium Report of this program, the testing methods they used or the form of measurement uncertainty of testing results they reported were corrected in this final report.

APPENDIX A

Summary of Testing Results

The testing results on nitrofuran metabolites contents obtained from the participants with corresponding testing methods and the individual z-scores are listed in Table A.1~A.4.

In these tables, some abbreviations and symbols were used. The symbols z_A, z_B and z_C denote individual z-scores of result A, result B, result C listed in the same table, respectively. The symbol "#" indicates a z-score derived from a questionable result, while "$" follows a z-score derived from an unsatisfactory result.

Table A.1 Testing Results with Their Assessments of AOZ in Samples A, B and C

LabCode	Result A (μ g/ kg)	z_A	Result B (μ g/ kg)	z_B	Result C (μ g/ kg)	z_C	Testing Method
T050-001	3.8±0.2	-0.32	6.7±0.2	-0.33	11.7±0.2	0.00	LC/MS/MS
T050-002	3.7±0.2	-0.39	8.4±0.2	0.37	12.5±0.2	0.15	LC/MS/MS
T050-003	2.28±0.10	-1.39	5.72±0.44	-0.73	5.88±0.98	-1.12	LC/MSn
T050-004	2.20	-1.45	4.32	-1.30	5.09	-1.28	LC/MSn
T050-005	4.5±0.5	0.17	6.8±0.7	-0.29	11.5±1.0	-0.04	LC/MS
T050-006	4.26±0.54	0.00	7.34±0.94	-0.07	12.08±1.55	0.07	LC/MSn
T050-007	5.44 ± 0.51	0.83	8.27 ± 0.51	0.32	16.4 ± 0.51	0.91	LC/MS/MS
T050-008	4.808±0.235	0.39	6.453±0.235	-0.43	8.862±0.235	-0.55	ELISA
T050-010	4.13±0.45	-0.09	7.82±0.61	0.13	11.80±2.68	0.02	ELISA
T050-011	5.03±0.66	0.54	9.04±2.06	0.63	12.34±1.42	0.12	ELISA
T050-012	6.5±0.5	1.57	11.8±1	1.76	20.2±2	1.64	LC/MSn
T050-013	3.38±0.28	-0.62	11.6±0.95	1.68	18.4±1.51	1.29	LC/MSn
T050-014	5.7±0.3	1.01	9.2±0.4	0.70	15.4±0.7	0.71	LC/MS/MS
T050-015	6.2±1.81	1.36	11.1±3.22	1.48	18.8±5.44	1.37	LC/MS/MS
T050-016	4.21±0.48	-0.04	7.51±1.00	0.00	9.56±0.84	-0.41	LC/MS/MS
T050-017	1.8±0.9	-1.73	2.9±1.5	-1.89	5.0±2.5	-1.29	LC/MSn
T050-018	5±1	0.52	7±2	-0.21	12±3	0.06	LC/MS/MS
T050-019	3.08	-0.83	4.10	-1.39	5.97	-1.11	LC/MSn
T050-020	4.97±0.60	0.50	9.01±0.47	0.62	13.83±0.94	0.41	LCMS
T050-021	4.861±0.778	0.42	8.033±1.285	0.22	10.402±1.664	-0.25	LC/MSn
T050-022	4.06	-0.14	7.32	-0.07	12.53	0.16	LC/MSn
T050-023	7.41	2.21#	12.3	1.97	20.6	1.72	LC/MS/MS
T050-024	4.0±0.80	-0.18	7.6±1.5	0.04	12.6±2.5	0.17	LC/MSn
T050-025	1.3±0.07	-2.08#	2.3±0.12	-2.13#	2.8±0.14	-1.72	LC/MSn
T050-026	4.9	0.45	7.5	0.00	12.0	0.06	LC/MS/MS
T050-027	5.0±11%	0.52	10.9±11%	1.39	8.7±11%	-0.58	LC/MS/MS
T050-028	2.21±0.25	-1.44	3.76±0.25	-1.53	6.01±0.25	-1.10	LC/MSn
T050-029	3.7±20%	-0.39	5.8±20%	-0.70	7.2±20%	-0.87	LC/MSn
T050-030	2.77	-1.05	4.30	-1.31	5.89	-1.12	ELISA
T050-031	5.50±0.15	0.87	8.07±0.22	0.23	8.31±0.22	-0.65	ELISA
T050-032	1.4±0.14	-2.01#	2.7±0.27	-1.97	4.4±0.44	-1.41	
T050-033	0.23±21%	-2.83#	0.51±21%	-2.87#	0.84±21%	-2.10#	LC/MSn
T050-034	2.80	-1.03	2.74	-1.95	0.62	-2.14#	LC/MSn
T050-035	4,74 ± 1,03	0.34	11,76 ± 2,56	1.75	26,72 ± 5,81	2.90#	UPLC/MS/MS
T050-037	5±1	0.52	8±1	0.21	11±2	-0.14	LC/MS/MS
T050-038	5.58±0.64	0.93	9.17±0.66	0.68	15.2±0.99	0.68	LC/MSn
T050-039	4.5±1.6	0.17	7.4±2.7	-0.04	13±4.7	0.25	

Table A.2 Testing Results with Their Assessments of AMOZ on Samples A, B and C

LabCode	Result A (μ g/ kg)	z_A	Result B (μ g/ kg)	z_B	Result C (μ g/ kg)	z_C	Testing Method
T050-001	2.4±0.2	-1.27	5.6±0.2	-0.83	9.2±0.2	-0.49	LC/MS/MS
T050-002	3.8±0.2	-0.40	7.9±0.2	0.00	12.4±0.2	0.19	LC/MS/MS
T050-003	3.79±0.20	-0.41	8.06±0.67	0.06	8.37±0.44	-0.66	LC/MSn
T050-004	2.18	-1.41	4.48	-1.24	5.40	-1.29	LC/MSn
T050-005	4.3±0.4	-0.09	6.8±0.7	-0.40	10.8±1.0	-0.15	LC/MS
T050-006	4.65±0.52	0.13	8.09±0.90	0.07	13.80±1.53	0.49	LC/MSn
T050-007	8.18 ± 0.49	2.33#	11.7 ± 0.49	1.39	16.03 ± 0.49	0.96	LC/MS/MS
T050-008	4.207±0.232	-0.15	7.500±0.232	-0.14	9.691±0.232	-0.38	ELISA
T050-010	3.58±0.30	-0.54	5.73±1.49	-0.79	10.64±2.60	-0.18	ELISA
T050-011	4.12±0.92	-0.20	9.39±1.42	0.55	13.65±1.72	0.45	ELISA
T050-012	0.5±0.05	-2.46#	4.6±0.5	-1.20	11.7±1	0.04	LC/MSn
T050-013	4.41±0.27	-0.02	7.61±0.47	-0.10	11.3±0.70	-0.04	LC/MSn
T050-014	5.4±0.3	0.60	9.2±0.5	0.48	15.0±0.8	0.74	LC/MS/MS
T050-015	9.1±1.67	2.91#	16.1±2.98	2.99#	27.0±5.02	3.28$	LC/MS/MS
T050-016	5.30±0.80	0.54	8.96±2.00	0.39	11.7±2.22	0.04	LC/MS/MS
T050-017	4.8±2.4	0.22	7.5±3.8	-0.14	9.8±3.1	-0.36	LC/MSn
T050-018	5±1	0.35	9±1	0.40	16±2	0.95	LC/MS/MS
T050-019	3.83	-0.38	4.18	-1.35	4.65	-1.45	LC/MSn
T050-020	5.59±0.27	0.72	8.55±0.17	0.24	15.38±0.49	0.82	LC/MS
T050-021	4.470±0.939	0.02	7.450±1.564	-0.16	9.572±2.010	-0.41	LC/MSn
T050-022	4.85	0.26	7.88	0.00	15.24	0.79	LC/MSn
T050-023	6.33	1.18	9.32	0.52	14.8	0.70	LC/MS/MS
T050-024	5.6±1.1	0.72	8.6±1.7	0.26	15.1±3.0	0.76	LC/MSn
T050-025	2.1±0.11	-1.46	3.5±0.18	-1.60	4.3±0.22	-1.52	LC/MSn
T050-026	6.8	1.47	9.3	0.51	13.4	0.40	LC/MS/MS
T050-027	6.85±13%	1.50	11.4±13%	1.28	12.6±13%	0.23	LC/MS/MS
T050-028	1.44±0.25	-1.87	2.25±0.25	-2.06#	4.14±0.25	-1.56	LC/MSn
T050-029	4.3±20%	-0.09	7.4±20%	-0.18	8.5±20%	-0.63	LC/MSn
T050-030	1.78	-1.66	2.95	-1.80	3.97	-1.59	ELISA
T050-032	1.2±0.1	-2.02#	2.2±0.18	-2.07#	3.6±0.29	-1.67	
T050-033	0.21±19%	-2.64#	0.46±19%	-2.71#	0.73±19%	-2.28#	LC/MSn
T050-034	1.64	-1.75	1.60	-2.29#	0.79	-2.26#	LC/MSn
T050-035	7,97 ± 0,81	2.20#	11,01 ± 1,12	1.14	29,72 ± 3,01	3.85$	UPLC/MS/MS
T050-037	5±1	0.35	8±2	0.04	11±2	-0.11	LC/MS/MS
T050-038	5.61±0.25	0.73	9.48±0.54	0.58	16.1±0.56	0.97	LC/MSn
T050-039	5.2±1.4	0.47	8.6±2.2	0.26	14±3.6	0.53	

Table A.3 Testing Results with Their Assessments of AHD on Samples A, B and C

LabCode	Result A (μ g/ kg)	z_A	Result B (μ g/ kg)	z_B	Result C (μ g/ kg)	z_C	Testing Method
T050-001	2.4±0.2	-1.18	4.5±0.2	-1.11	7.9±0.2	-0.86	LC/MS/MS
T050-002	4.5±0.2	0.28	8.8±0.2	0.66	14.1±0.2	0.86	LC/MS/MS
T050-003	3.41±0.18	-0.48	9.70±1.35	1.03	8.62±0.77	-0.66	LC/MSn
T050-004	2.62	-1.02	4.97	-0.92	5.63	-1.50	LC/MSn
T050-005	4.1±0.4	0.00	5.6±0.5	-0.66	10.2±1.0	-0.22	LC/MS
T050-006	5.71±0.72	1.11	9.15±1.15	0.80	19.64±2.47	2.41#	LC/MSn
T050-007	4.90 ± 0.5	0.55	8.40 ± 0.5	0.49	15.8 ± 0.5	1.34	LC/MS/MS
T050-012	3.5±0.3	-0.42	7.1±0.5	-0.04	11.4±1	0.11	LC/MSn
T050-013	3.68±0.77	-0.29	5.72±1.20	-0.61	9.54±2.00	-0.41	LC/MSn
T050-014	3.3±0.4	-0.55	6.3±0.7	-0.37	9.5±1.0	-0.42	LC/MS/MS
T050-015	4.6±1.38	0.35	8.6±2.54	0.58	14.0±4.18	0.84	LC/MS/MS
T050-016	5.92±0.31	1.26	8.96±1.56	0.72	13.2±1.31	0.61	LC/MS/MS
T050-017	7.0±3.5	2.01#	7.2±3.6	0.00	13.3±4.3	0.64	LC/MSn
T050-018	4±1	-0.07	8±2	0.33	13±2	0.56	LC/MS/MS
T050-019	1.84	-1.56	2.20	-2.06#	3.41	-2.12#	LC/MSn
T050-020	4.39±0.72	0.20	8.63±0.75	0.59	12.13±2.01	0.31	LC/MS
T050-021	5.263±0.947	0.80	8.808±1.585	0.66	11.353±2.043	0.10	LC/MSn
T050-022	4.19	0.06	8.53	0.55	13.57	0.72	LC/MSn
T050-023	4.41	0.21	6.55	-0.27	12.0	0.28	LC/MS/MS
T050-024	4.5±0.9	0.28	7.4±1.5	0.08	12.2±2.4	0.33	LC/MSn
T050-025	2.7±0.14	-0.97	4.6±0.23	-1.07	5.5±0.28	-1.53	LC/MSn
T050-026	4.9	0.55	7.5	0.12	11.7	0.20	LC/MS/MS
T050-027	5.7±14%	1.11	8.0±14%	0.33	9.6±14%	-0.39	LC/MS/MS
T050-028	2.53±0.25	-1.09	4.69±0.25	-1.03	7.46±0.25	-0.99	LC/MSn
T050-029	1.8±20%	-1.59	2.5±20%	-1.93	3.1±20%	-2.20#	LC/MSn
T050-032	3.2±0.45	-0.62	6.5±0.91	-0.29	9.5±1.33	-0.42	
T050-033	0.21±23%	-2.69#	0.49±23%	-2.76#	0.76±23%	-2.85#	LC/MSn
T050-034	0.70	-2.35#	2.74	-1.83	1.07	-2.77#	LC/MSn
T050-037	6±1	1.31	9±2	0.74	11±2	0.00	LC/MS/MS
T050-038	5.02±0.34	0.64	8.24±0.56	0.43	14.5±0.70	0.98	LC/MSn
T050-039	3.8±1.6	-0.21	6.3±2.7	-0.37	11±4.7	0.00	

Table A.4 Testing Results with Their Assessments of SEM on Samples A, B and C

LabCode	Result A (μg/kg)	z_A	Result B (μg/kg)	z_B	Result C (μg/kg)	z_C	Testing Method
T050-001	2.9±0.2	-0.95	5.6±0.2	-0.59	9.0±0.2	-0.83	LC/MS/MS
T050-002	6.3±0.2	0.59	11.3±0.2	1.07	16.7±0.2	0.98	LC/MS/MS
T050-003	7.52±0.47	1.14	12.36±1.48	1.37	15.17±1.37	0.62	LC/MSn
T050-004	2.39	-1.18	4.66	-0.87	5.53	-1.64	LC/MSn
T050-005	5.0±0.5	0.00	16.0±1.6	2.43#	10.8±1.0	-0.41	LC/MS
T050-006	4.02±0.45	-0.44	6.53±0.73	-0.32	12.54±1.40	0.00	LC/MSn
T050-007	6.44 ± 0.48	0.65	12.6 ± 0.48	1.44	19.46 ± 0.48	1.62	LC/MS/MS
T050-012	6.7±0.5	0.77	12.1±1	1.30	20.0±2	1.75	LC/MSn
T050-013	9.27±0.66	1.94	14.2±1.02	1.91	23.7±1.68	2.62#	LC/MSn
T050-014	3.3±0.5	-0.77	6.3±0.9	-0.39	9.0±1.3	-0.83	LC/MS/MS
T050-015	5.1±0.87	0.05	8.9±1.52	0.37	15.0±2.58	0.58	LC/MS/MS
T050-016	5.32±0.55	0.15	8.94±1.84	0.38	14.4±1.60	0.44	LC/MS/MS
T050-017	5.9±3.0	0.41	8.3±4.2	0.19	15.6±5.0	0.72	LC/MSn
T050-018	5±1	0.00	8±2	0.10	13±3	0.11	LC/MS/MS
T050-019	1.88	-1.41	3.26	-1.27	2.64	-2.32#	LC/MSn
T050-020	2.75±0.29	-1.02	5.73±0.33	-0.56	7.85±0.86	-1.10	LC/MS
T050-021	4.449±0.801	-0.25	7.148±1.267	-0.14	9.251±1.665	-0.77	LC/MSn
T050-022	4.18	-0.37	7.11	-0.15	11.01	-0.36	LC/MSn
T050-023	5.04	0.02	7.64	0.00	14.2	0.39	LC/MS/MS
T050-024	12.8±2.6	3.54$	17.1±3.4	2.75#	19.0±3.8	1.52	LC/MSn
T050-025	2.2±0.11	-1.27	4.0±0.20	-1.06	4.4±0.22	-1.91	LC/MSn
T050-026	5.2	0.09	7.2	-0.13	10.4	-0.50	LC/MS/MS
T050-027	6.5±16%	0.68	10.0±16%	0.69	10.7±16%	-0.43	LC/MS/MS
T050-028	2.95±0.25	-0.93	3.24±0.25	-1.28	7.92±0.25	-1.08	LC/MSn
T050-029	6.6±20%	0.73	12.2±20%	1.33	12.9±20%	0.08	LC/MSn
T050-032	4.3±0.52	-0.32	8.2±0.98	0.16	13.7±1.64	0.27	
T050-033	0.60±32%	-2.00	1.39±32%	-1.82	1.94±32%	-2.49#	LC/MSn
T050-034	1.72	-1.49	1.08	-1.91	2.22	-2.42#	LC/MSn
T050-037	5±1	0.00	7±2	-0.19	10±3	-0.60	LC/MS/MS
T050-038	5.18±0.18	0.08	8.23±0.24	0.17	14.5±0.68	0.46	LC/MSn
T050-039	4.7±3.0	-0.14	7.2±4.6	-0.13	13±8.3	0.11	

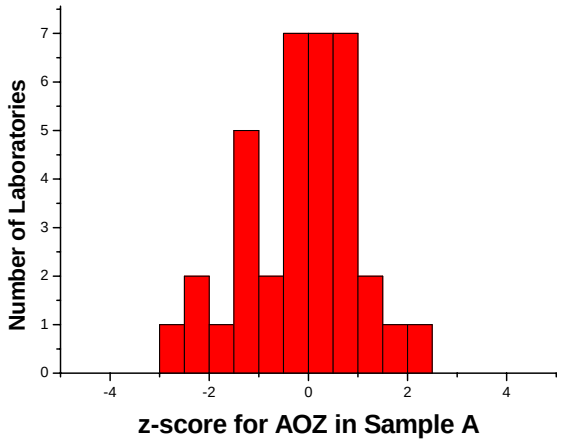
APPENDIX B

Graphical Display of Testing Results

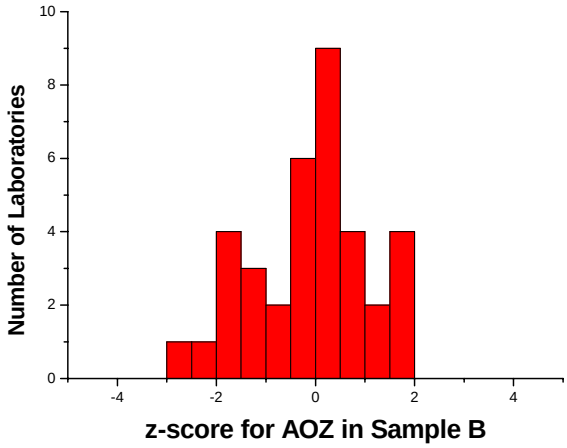
Here, graphs were made in order to give an open-and-shut display of testing results and their statistics.

Histograms of z-scores (B.1~B.12) show the distributions of testing results. Ordered z-score bar charts (B.13~B.24) are given in order to facilitate visual identification of the questionable and unsatisfactory results.

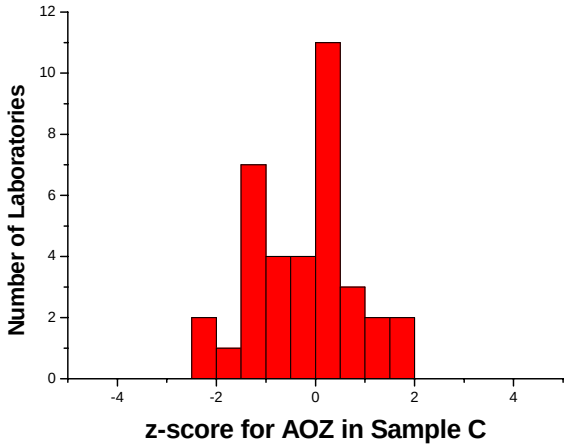
B.1 Distribution of Testing Results of AOZ in Sample A



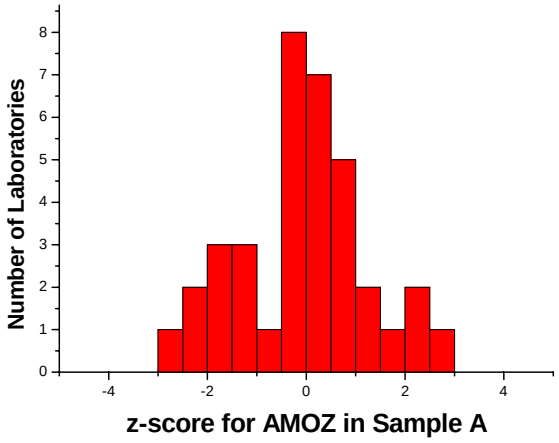
B.2 Distribution of Testing Results of AOZ in Sample B



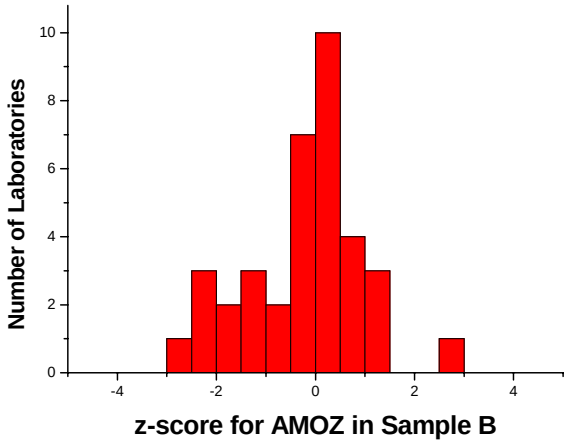
B.3 Distribution of Testing Results of AOZ in Sample C



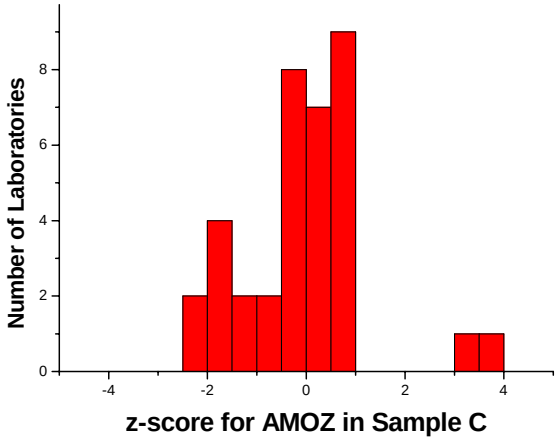
B.4 Distribution of Testing Results of AMOZ in Sample A



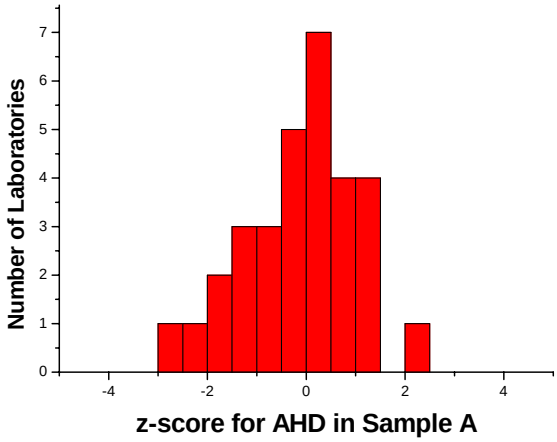
B.5 Distribution of Testing Results of AMOZ in Sample B



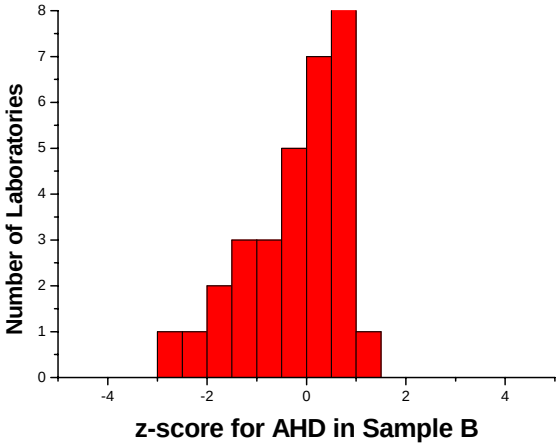
B.6 Distribution of Testing Results of AMOZ in Sample C



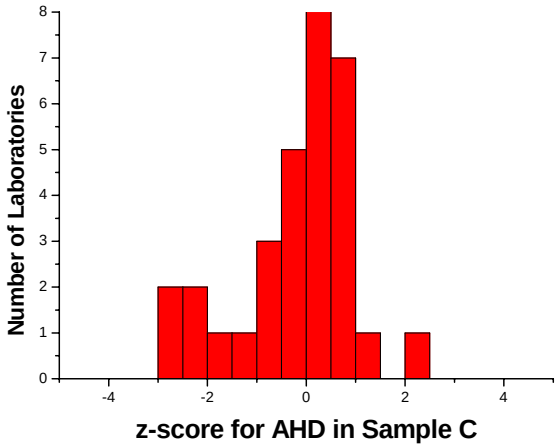
B.7 Distribution of Testing Results of AHD in Sample A



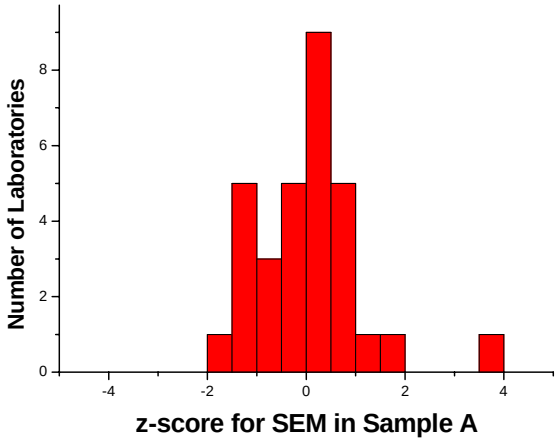
B.8 Distribution of Testing Results of AHD in Sample B



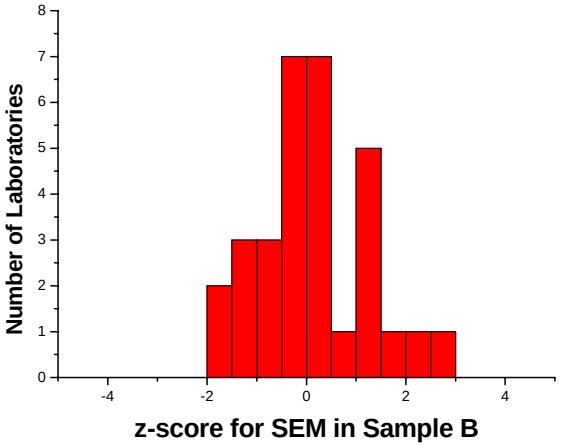
B.9 Distribution of Testing Results of AHD in Sample C



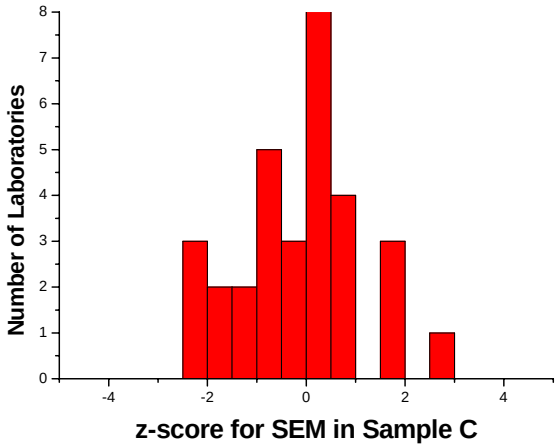
B.10 Distribution of Testing Results of SEM in Sample A



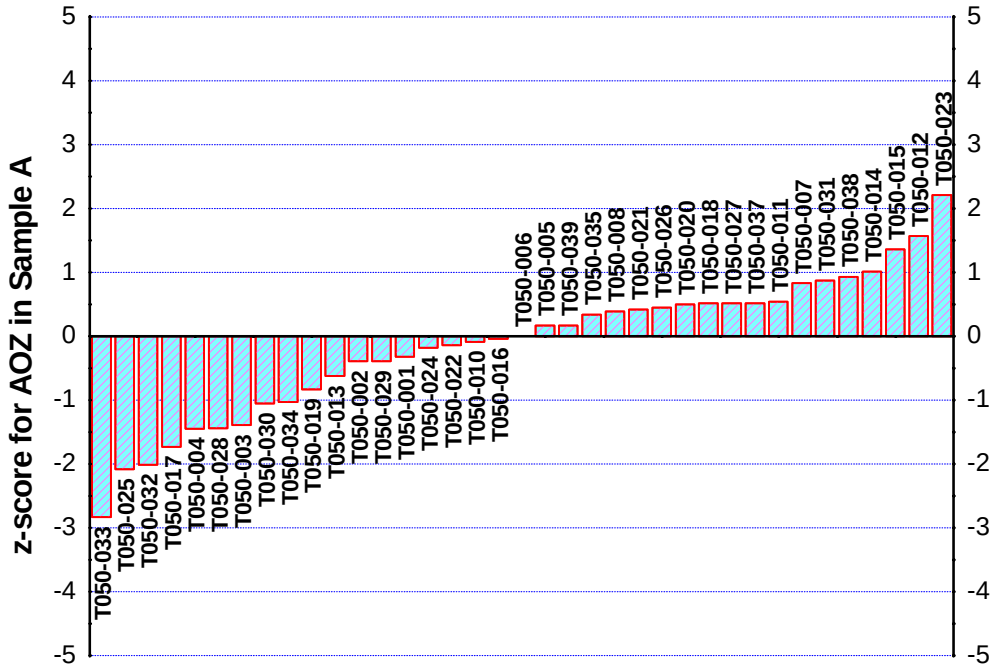
B.11 Distribution of Testing Results of SEM in Sample B



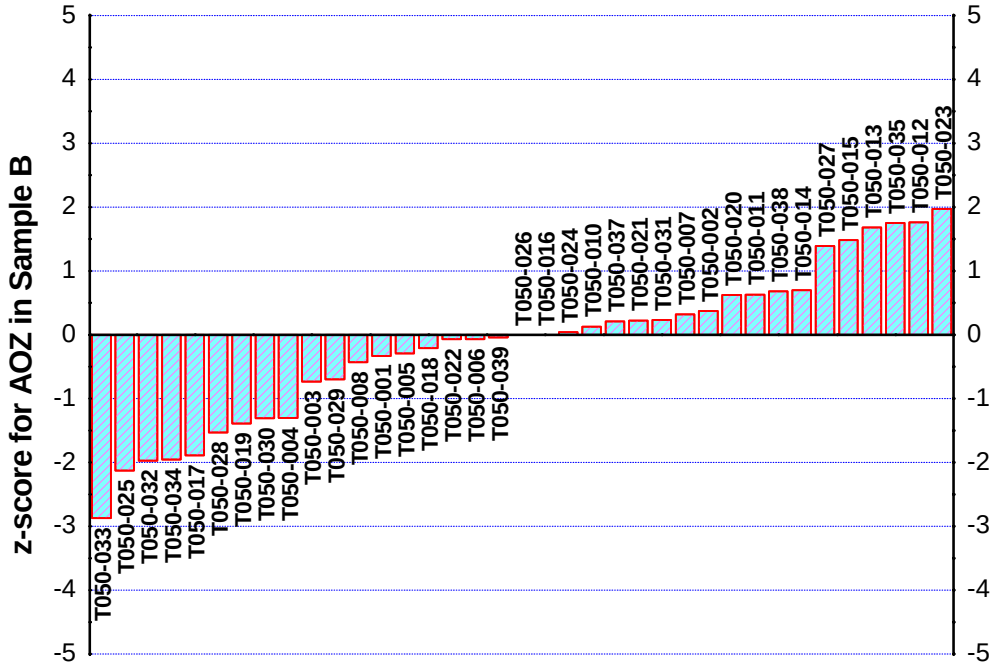
B.12 Distribution of Testing Results of SEM in Sample C



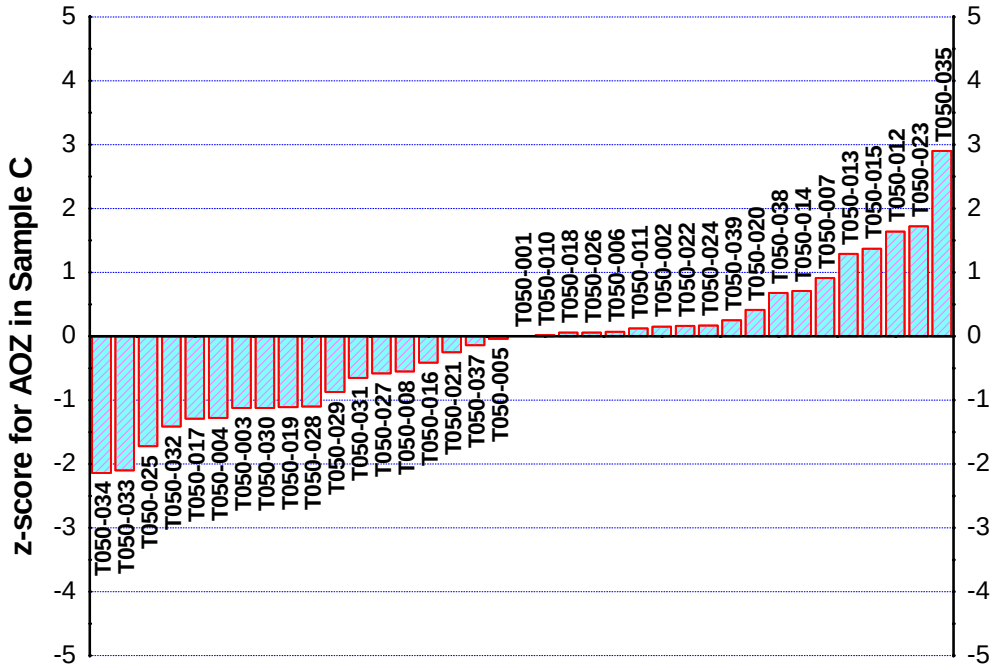
B.13 Ordered z-scores Bar Chart of Results of AOZ in Sample A



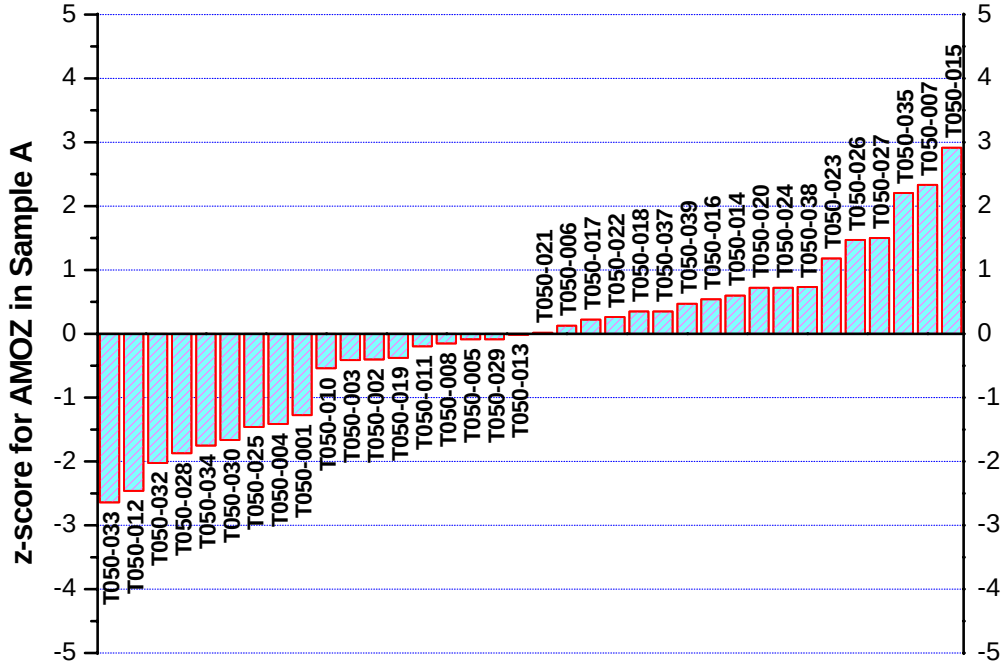
B.14 Ordered z-scores Bar Chart of Results of AOZ in Sample B



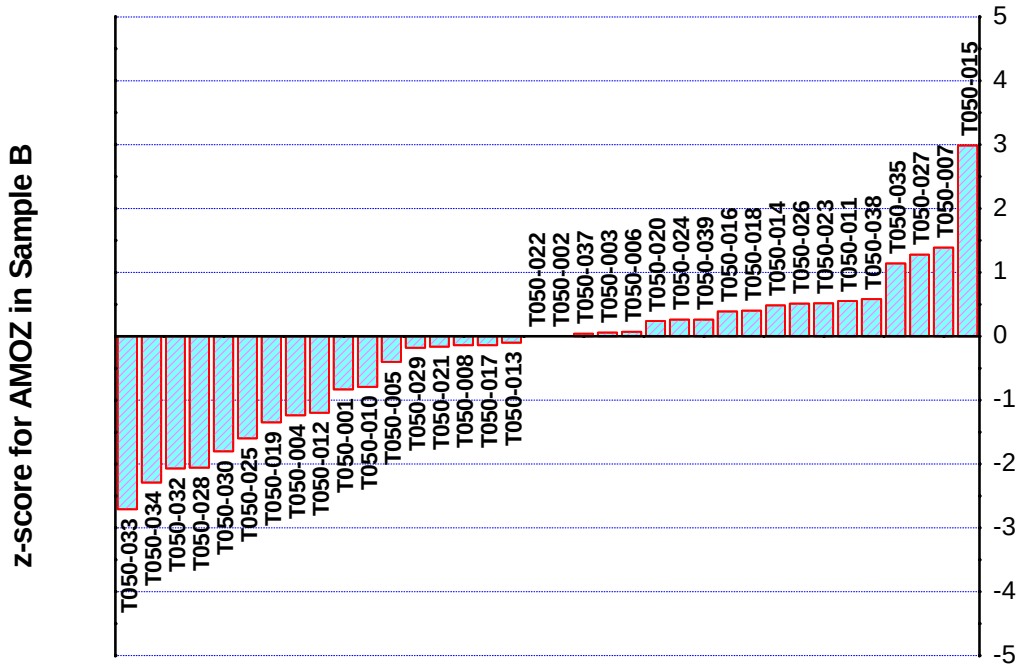
B.15 Ordered z-scores Bar Chart of Results of AOZ in Sample C



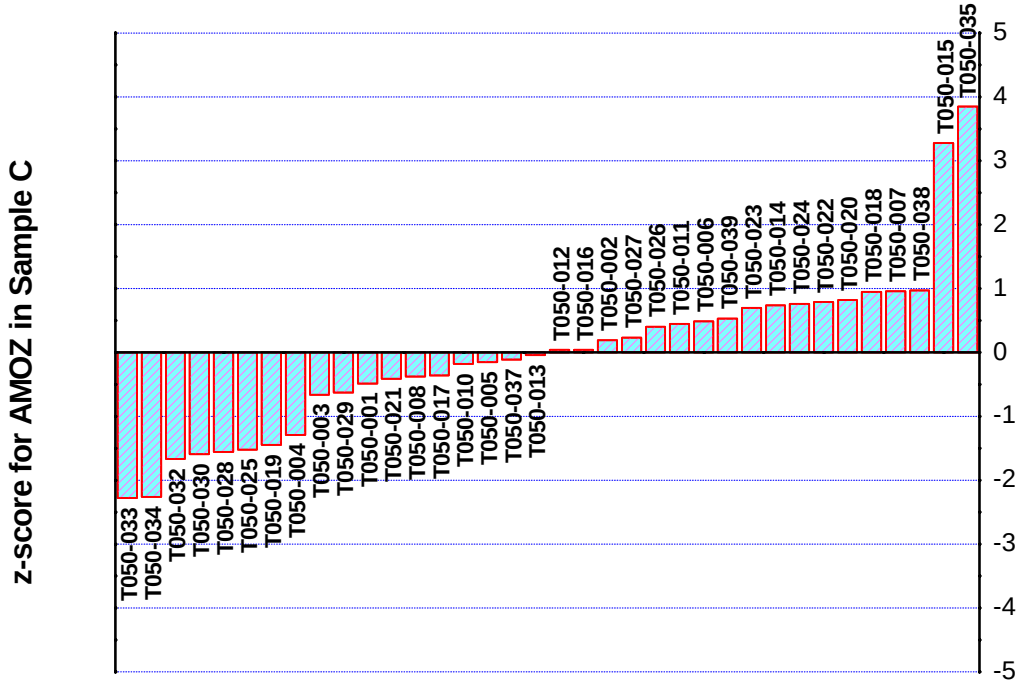
B.16 Ordered z-scores Bar Chart of Results of AMOZ in Sample A



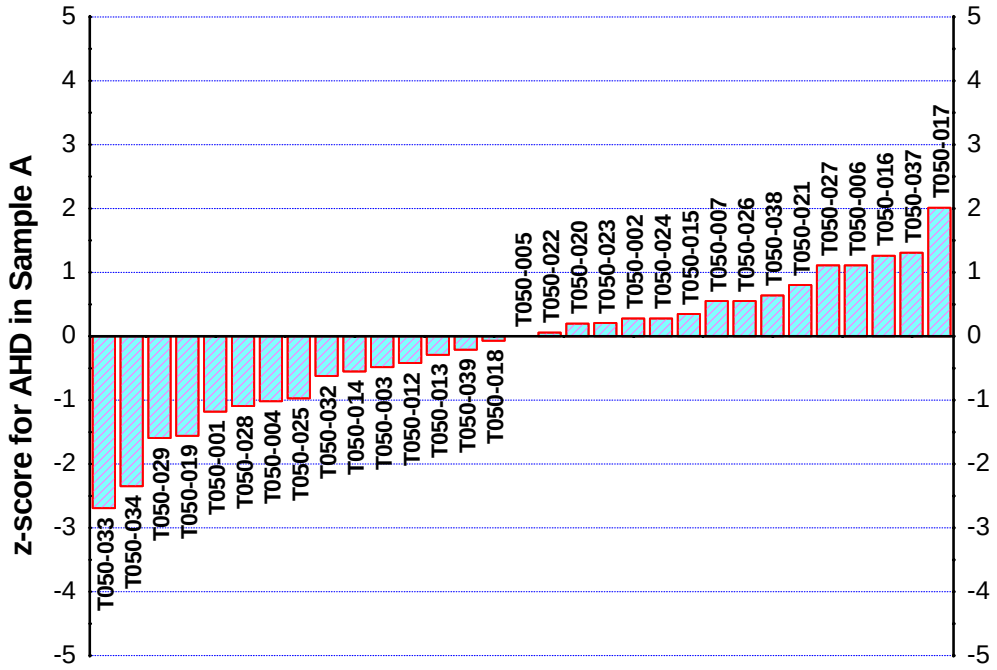
B.17 Ordered z-scores Bar Chart of Results of AMOZ in Sample B



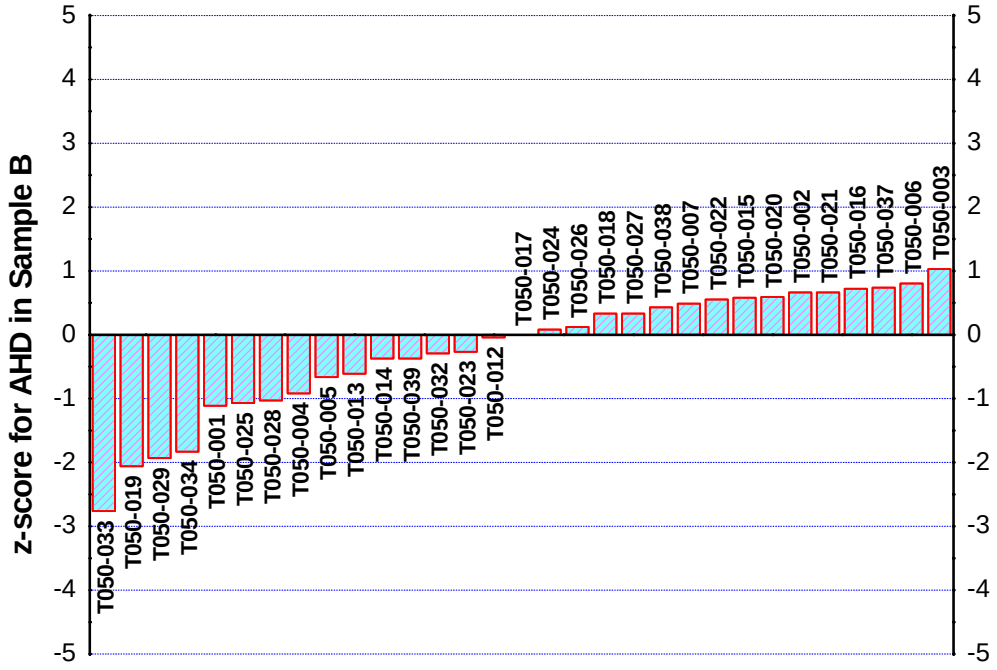
B.18 Ordered z-scores Bar Chart of Results of AMOZ in Sample C



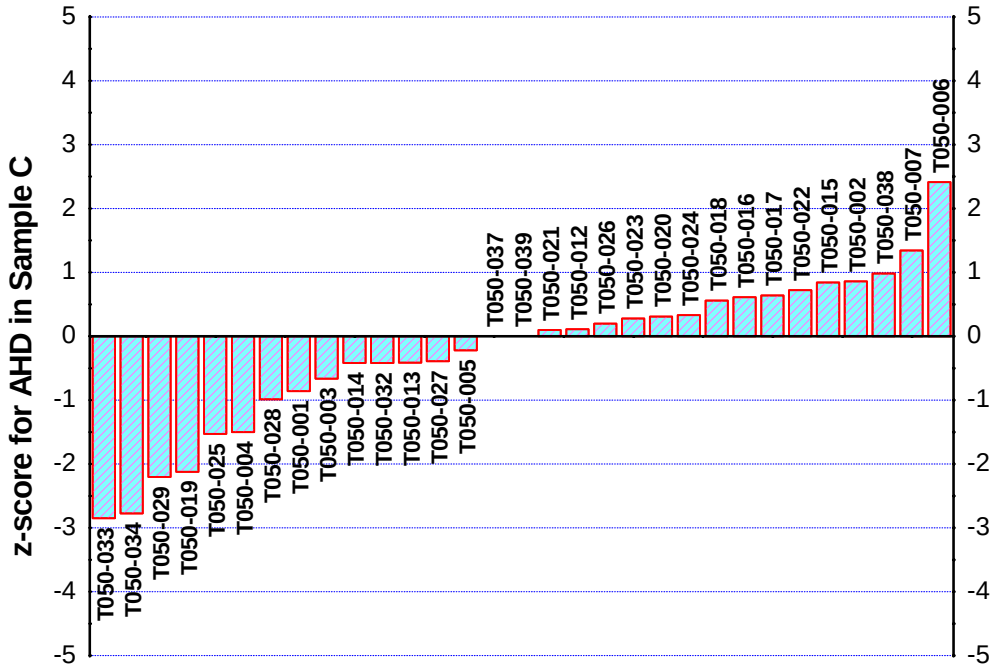
B.19 Ordered z-scores Bar Chart of Results of AHD in Sample A



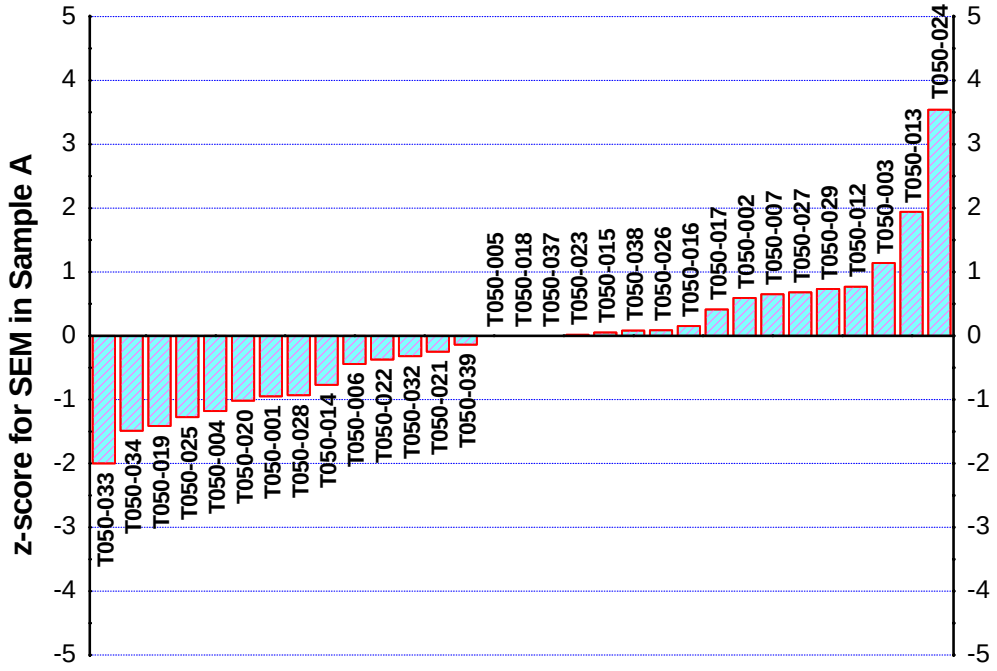
B.20 Ordered z-scores Bar Chart of Results of AHD in Sample B



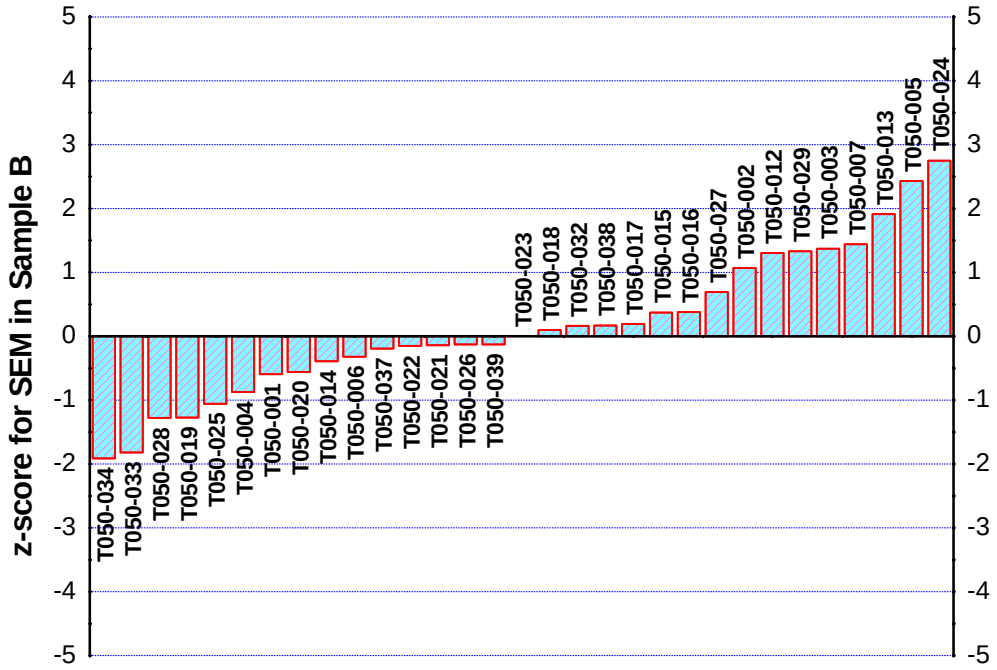
B.21 Ordered z-scores Bar Chart of Results of AHD in Sample C



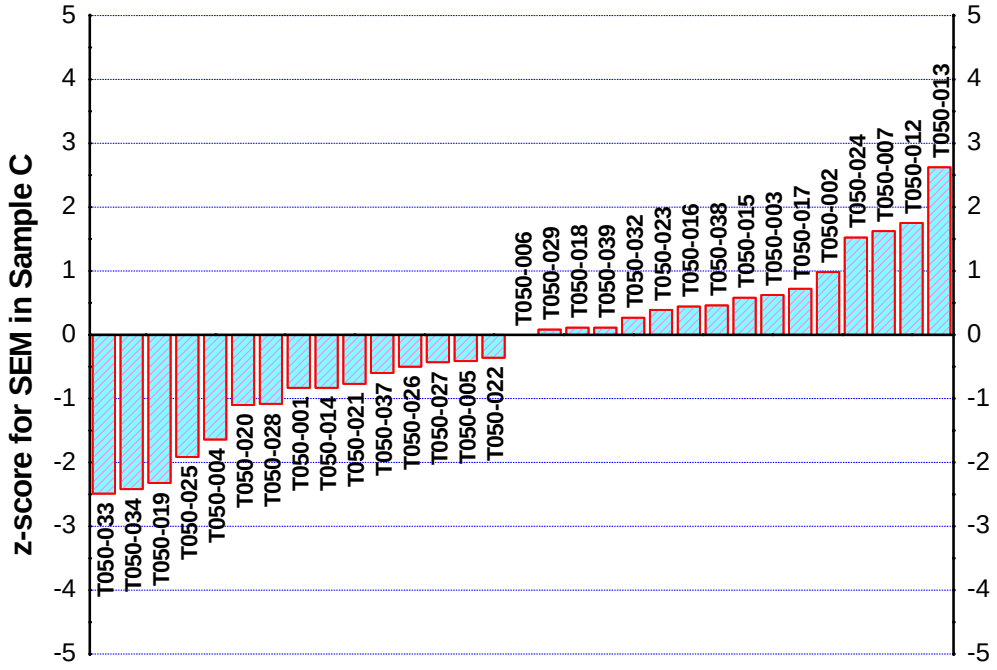
B.22 Ordered z-scores Bar Chart of Results of SEM in Sample A



B.23 Ordered z-scores Bar Chart of Results of SEM in Sample B



B.24 Ordered z-scores Bar Chart of Results of SEM in Sample C



APPENDIX C

Homogeneity and Stability Checks

C.1 Homogeneity Testing

For each gross sample, 12 samples were randomly selected and tested. Each sample was divided into two test portions, all test portions were tested in random order on the repeatability condition, that is to say, the testing was carried out during a short period by one person using the same testing method and apparatus in one laboratory. The results on each sample are listed in Table C.1~C.12.

The **between-samples standard deviations (s_s)** were calculated using the following formulae, where g is the number of samples used:

The **sample average** is $\bar{x}_t = (x_{t1} + x_{t2})/2$

The **between-test-portion range** is $w_t = | x_{t1} - x_{t2} |$

General average is $\bar{\bar{x}} = \sum \bar{x}_t / g$

The standard deviation of general average is $s_x = \sqrt{\sum (\bar{x}_t - \bar{\bar{x}})^2 / (g-1)}$

The within-sample standard deviation is $s_w = \sqrt{\sum w_t^2 / 2g}$

The between-samples standard deviation is $s_s = \sqrt{s_x^2 - s_w^2 / 2}$

Table C.1 Homogeneity Testing Results of AOZ in Sample A

Sample	x_{t1}(μ g/kg)	x_{t2}(μ g/kg)	$\bar{x}_t$ (μ g/kg)	w_t(μ g/kg)	w_t^2, (μ g/kg)^2
1	4.41	4.24	4.33	0.17	0.0289
2	4.68	4.62	4.65	0.06	0.0036
3	4.68	4.76	4.72	0.08	0.0064
4	4.62	4.16	4.39	0.46	0.2116
5	4.5	4.42	4.46	0.08	0.0064
6	4.54	4.42	4.48	0.12	0.0144
7	4.30	4.49	4.40	0.19	0.0361
8	4.67	4.57	4.62	0.10	0.0100
9	4.37	4.43	4.40	0.06	0.0036
10	4.71	4.72	4.72	0.01	0.0001
11	4.51	4.68	4.60	0.17	0.0289
12	4.38	4.20	4.29	0.18	0.0324
$\bar{\bar{x}}$ =4.50 μ g/kg, NIQR=1.4 μ g/kg, s_s=0.12 μ g/kg, s_s/NIQR=0.09<0.3					

Table C.2 Homogeneity Testing Results of AOZ in Sample B

Sample	x_{t1}(μ g/kg)	x_{t2}(μ g/kg)	$\bar{x}_t$ (μ g/kg)	w_t(μ g/kg)	w_t^2, (μ g/kg)^2
1	8.15	8.14	8.15	0.01	0.0001
2	8.12	8.12	8.12	0	0
3	7.74	7.74	7.74	0	0
4	8.03	7.99	8.01	0.04	0.0016
5	7.77	7.54	7.66	0.23	0.0529
6	7.97	7.91	7.94	0.06	0.0036
7	7.66	7.6	7.63	0.06	0.0036
8	7.41	7.59	7.50	0.18	0.0324
9	7.56	7.60	7.58	0.04	0.0016
10	7.54	7.62	7.58	0.08	0.0064
11	7.4	7.55	7.48	0.15	0.0225
12	7.98	7.88	7.93	0.10	0.0100
$\bar{\bar{x}}$ =7.78 μ g/kg, NIQR=2.4 μ g/kg, s_s=0.24 μ g/kg, s_s/NIQR=0.10<0.3					

Table C.3 Homogeneity Testing Results of AOZ in Sample C

Sample	x_{t1}(μ g/kg)	x_{t2}(μ g/kg)	$\bar{x}_t$ (μ g/kg)	w_t(μ g/kg)	w_t^2, (μ g/kg)^2
1	11.9	11.9	11.90	0	0
2	11.4	11.7	11.55	0.3	0.09
3	11.9	12.0	11.95	0.1	0.01
4	11.8	12.1	11.95	0.3	0.09
5	11.5	11.5	11.50	0	0
6	11.8	11.9	11.85	0.1	0.01
7	11.4	11.4	11.40	0	0
8	11.6	11.4	11.50	0.2	0.04
9	11.5	11.3	11.40	0.2	0.04
10	11.6	11.6	11.60	0	0
11	11.6	11.5	11.55	0.1	0.01
12	11.6	11.7	11.65	0.1	0.01
$\bar{\bar{x}}$ =11.65 μ g/kg, NIQR=5.2 μ g/kg, s_s=0.19 μ g/kg, s_s/NIQR=0.04<0.3					

Table C.4 Homogeneity Testing Results of AMOZ in Sample A

Sample	x_{t1}(μ g/kg)	x_{t2}(μ g/kg)	$\bar{x}_t$ (μ g/kg)	w_t(μ g/kg)	w_t^2, (μ g/kg)^2
1	4.50	4.53	4.52	0.03	0.0009
2	4.43	4.37	4.40	0.06	0.0036
3	4.27	4.29	4.28	0.02	0.0004
4	4.35	4.44	4.40	0.09	0.0081
5	4.52	4.47	4.50	0.05	0.0025
6	4.42	4.35	4.39	0.07	0.0049
7	4.34	4.31	4.33	0.03	0.0009
8	4.48	4.42	4.45	0.06	0.0036
9	4.62	4.48	4.55	0.14	0.0196
10	4.37	4.42	4.40	0.05	0.0025
11	4.31	4.40	4.36	0.09	0.0081
12	4.45	4.42	4.44	0.03	0.0009
$\bar{\bar{x}}$ =4.42 μ g/kg, NIQR=1.6 μ g/kg, s_s=0.07 μ g/kg, s_s/NIQR=0.04<0.3					

Table C.5 Homogeneity Testing Results of AMOZ in Sample B

Sample	x_{t1}(μ g/kg)	x_{t2}(μ g/kg)	$\bar{x}_t$ (μ g/kg)	w_t(μ g/kg)	w_t^2, (μ g/kg)^2
1	8.05	7.94	8.00	0.11	0.0121
2	8.03	8.17	8.10	0.14	0.0196
3	8.01	8.22	8.12	0.21	0.0441
4	7.78	7.62	7.70	0.16	0.0256
5	7.97	8.12	8.05	0.15	0.0225
6	8.03	8.15	8.09	0.12	0.0144
7	8.17	8.04	8.11	0.13	0.0169
8	7.92	8.04	7.98	0.12	0.0144
9	8.07	8.14	8.11	0.07	0.0049
10	8.08	8.01	8.05	0.07	0.0049
11	8.38	8.44	8.41	0.06	0.0036
12	8.37	8.28	8.33	0.09	0.0081
$\bar{\bar{x}}$ =8.08 μ g/kg, NIQR=2.7 μ g/kg, s_s=0.16 μ g/kg, s_s/NIQR=0.15<0.3					

Table C.6 Homogeneity Testing Results of AMOZ in Sample C

Sample	x_{t1}(μ g/kg)	x_{t2}(μ g/kg)	$\bar{x}_t$ (μ g/kg)	w_t(μ g/kg)	w_t^2, (μ g/kg)^2
1	11.80	11.80	11.80	0	0
2	11.30	11.50	11.40	0.2	0.04
3	11.50	11.60	11.55	0.1	0.01
4	11.90	11.80	11.85	0.1	0.01
5	11.00	10.50	10.75	0.5	0.25
6	11.60	11.30	11.45	0.3	0.09
7	11.70	11.70	11.70	0	0
8	12.00	11.90	11.95	0.1	0.01
9	11.20	11.00	11.10	0.2	0.04
10	12.10	11.80	11.95	0.3	0.09
11	11.90	11.70	11.80	0.2	0.04
12	11.80	11.70	11.75	0.1	0.01
$\bar{\bar{x}}$ =11.59 μ g/kg, NIQR=4.7 μ g/kg, s_s=0.35 μ g/kg, s_s/NIQR=0.17<0.3					

Table C.7 Homogeneity Testing Results of AHD in Sample A

Sample	x_{t1}(μ g/kg)	x_{t2}(μ g/kg)	$\bar{x}_t$ (μ g/kg)	w_t(μ g/kg)	w_t^2, (μ g/kg)^2
1	3.90	3.92	3.91	0.02	0.0004
2	4.01	3.91	3.96	0.10	0.0100
3	3.90	4.29	4.10	0.39	0.1521
4	3.93	3.90	3.92	0.03	0.0009
5	3.93	4.15	4.04	0.22	0.0484
6	3.99	4.23	4.11	0.24	0.0576
7	4.29	4.31	4.30	0.02	0.0004
8	4.06	4.14	4.10	0.08	0.0064
9	4.45	4.5	4.48	0.05	0.0025
10	4.19	4.21	4.20	0.02	0.0004
11	4.36	4.38	4.37	0.02	0.0004
12	4.08	4.18	4.13	0.10	0.0100
$\bar{\bar{x}}$ =4.13 μ g/kg, NIQR=1.4 μ g/kg, s_s=0.16 μ g/kg, s_s/NIQR=0.11<0.3					

Table C.8 Homogeneity Testing Results of AHD in Sample B

Sample	x_{t1}(μ g/kg)	x_{t2}(μ g/kg)	$\bar{x}_t$ (μ g/kg)	w_t(μ g/kg)	w_t^2, (μ g/kg)^2
1	6.81	6.69	6.75	0.12	0.0144
2	7.15	7.29	7.22	0.14	0.0196
3	7.22	7.25	7.24	0.03	0.0009
4	7.12	6.91	7.02	0.21	0.0441
5	7.15	7.04	7.10	0.11	0.0121
6	6.96	6.91	6.94	0.05	0.0025
7	6.63	6.76	6.70	0.13	0.0169
8	6.57	6.88	6.73	0.31	0.0961
9	6.69	6.7	6.70	0.01	0.0001
10	6.54	6.43	6.49	0.11	0.0121
11	7.3	7.21	7.26	0.09	0.0081
12	7.14	6.83	6.99	0.31	0.0961
$\bar{\bar{x}}$ =6.92 μ g/kg, NIQR=2.4 μ g/kg, s_s=0.24 μ g/kg, s_s/NIQR=0.22<0.3					

Table C.9 Homogeneity Testing Results of AHD in Sample C

Sample	x_{t1}(μ g/kg)	x_{t2}(μ g/kg)	$\bar{x}_t$ (μ g/kg)	w_t(μ g/kg)	w_t^2, (μ g/kg)^2
1	10.2	9.94	10.07	0.26	0.0676
2	10.2	10.1	10.15	0.10	0.0100
3	10.1	10.1	10.10	0	0.0000
4	10.1	10.0	10.05	0.10	0.0100
5	9.52	9.44	9.48	0.08	0.0064
6	10.4	10.7	10.55	0.30	0.0900
7	10.1	9.53	9.82	0.57	0.3249
8	10.2	9.85	10.03	0.35	0.1225
9	10.5	9.49	10.00	1.01	1.0201
10	10.0	10.4	10.20	0.40	0.1600
11	10.5	10.4	10.45	0.10	0.0100
12	10.1	9.67	9.89	0.43	0.1849
$\bar{\bar{x}}$ =11.59 μ g/kg, NIQR=4.7 μ g/kg, s_s=0.35 μ g/kg, s_s/NIQR=0.17<0.3					

Table C.10 Homogeneity Testing Results of SEM in Sample A

Sample	x_{t1}(μ g/kg)	x_{t2}(μ g/kg)	$\bar{x}_t$ (μ g/kg)	w_t(μ g/kg)	w_t^2, (μ g/kg)^2
1	4.98	4.95	4.97	0.23	0.0529
2	5.04	5.04	5.04	0.06	0.0036
3	5.04	5.18	5.11	0	0.0000
4	4.93	4.82	4.88	0.11	0.0121
5	4.62	4.78	4.70	0.16	0.0256
6	4.86	4.75	4.81	0.11	0.0121
7	4.57	4.69	4.63	0.12	0.0144
8	5.09	4.84	4.97	0.25	0.0625
9	4.86	4.74	4.80	0.12	0.0144
10	4.76	4.68	4.72	0.08	0.0064
11	4.81	4.64	4.73	0.17	0.0289
12	4.87	4.75	4.81	0.12	0.0144
$\bar{\bar{x}}$ =4.85 μ g/kg, NIQR=2.2 μ g/kg, s_s=0.13 μ g/kg, s_s/NIQR=0.06<0.3					

Table C.11 Homogeneity Testing Results of SEM in Sample B

Sample	x_{t1}(μ g/kg)	x_{t2}(μ g/kg)	$\bar{x}_t$ (μ g/kg)	w_t(μ g/kg)	w_t^2, (μ g/kg)^2
1	8.08	7.96	8.02	0.2	0.0400
2	8.02	7.96	7.99	0.06	0.0036
3	7.79	7.76	7.78	0.17	0.0289
4	8.06	8.25	8.16	0.19	0.0361
5	7.81	7.86	7.84	0.05	0.0025
6	7.49	7.55	7.52	0.06	0.0036
7	7.64	7.41	7.53	0.23	0.0529
8	7.44	7.26	7.35	0.18	0.0324
9	8.08	8.06	8.07	0.02	0.0004
10	7.79	7.63	7.71	0.16	0.0256
11	7.14	7.35	7.25	0.21	0.0441
12	7.44	7.20	7.32	0.24	0.0576
$\bar{\bar{x}}$ =7.71 μ g/kg, NIQR=3.4 μ g/kg, s_s=0.30 μ g/kg, s_s/NIQR=0.20<0.3					

Table C.12 Homogeneity Testing Results of SEM in Sample C

Sample	x_{t1}(μ g/kg)	x_{t2}(μ g/kg)	$\bar{x}_t$ (μ g/kg)	w_t(μ g/kg)	w_t^2, (μ g/kg)^2
1	13.00	12.90	12.95	0.5	0.2500
2	13.20	12.90	13.05	0.2	0.0400
3	12.20	12.40	12.30	0.7	0.4900
4	12.40	13.00	12.70	0.6	0.3600
5	12.40	11.50	11.95	0.9	0.8100
6	12.30	12.10	12.20	0.2	0.0400
7	12.10	12.40	12.25	0.3	0.0900
8	12.60	12.80	12.70	0.2	0.0400
9	12.90	12.70	12.80	0.2	0.0400
10	12.10	11.80	11.95	0.3	0.0900
11	11.40	11.70	11.55	0.3	0.0900
12	12.30	11.40	11.85	0.9	0.8100
$\bar{\bar{x}}$ =12.35 μ g/kg, NIQR=4.3 μ g/kg, s_s=0.41 μ g/kg, s_s/NIQR=0.21<0.3					

Comparing the between-samples standard deviation (s_s) with the corresponding standard deviation for proficiency assessment (NIQR), all s_s are less than 0.3NIQR, it indicates that the variation between samples could be neglected in subsequently statistical analysis and proficiency assessments, in another word, the samples are homogenous enough.

C.2 Stability Testing

For Gross Sample A and C, 12 packaged testing samples were selected for stability testing, respectively. The samples were placed at room temperature (18~25 degrees C) for 2 days, then putted into an attemperator with a temperature of 40 degrees C for 7 days and placed at about 4 degrees C until stability testing finished, on the date 10, 20, 55 and 100 beginning from the day of homogeneity testing, every 3 samples per date were tested on the contents of each metabolite concerned in this program.

For all stability testing, the person, apparatus, testing method and laboratory were same as the homogeneity testing. The testing results of stability check are listed in Table C.13~C.14, every absolute differences from the general average of homogeneity tests were calculated using following formulae:

The sample average is $\bar{y}_t = (y_{t1} + y_{t2})/2$

General average is $\bar{\bar{y}} = \sum \bar{y}_t / g$

The difference between stability and homogeneity testing results is $|d| = |\bar{\bar{y}} - \bar{\bar{x}}|$

Comparing with the standard deviations for proficiency assessment (NIQR) in this program, all maximal absolute differences between the stability testing results and the corresponding homogeneity testing results (|d|) for each residue content in each sample are less than 0.3NIQR, it indicates that the variation of samples could be neglected in subsequently statistical analysis and proficiency assessments.

As a conclusion, all samples are stable enough for the purposes of this program.

Table C.13 Stability Testing Results in Sample A

Unit: μg/kg

AOZ						AMOZ					
Day	y_{t1}	y_{t2}	$\bar{y}_t$	$\bar{\bar{y}}$	|d|	Day	y_{t1}	y_{t2}	$\bar{y}_t$	$\bar{\bar{y}}$	|d|
10	4.26	4.45	4.36	4.41	0.09	10	4.66	4.72	4.69	4.48	0.06
	4.22	4.56	4.39				4.43	4.39	4.41		
	4.52	4.47	4.50				4.28	4.37	4.33		
20	4.30	4.12	4.21	4.35	0.15	20	4.55	4.63	4.59	4.47	0.05
	4.60	4.33	4.47				4.32	4.49	4.41		
	4.48	4.28	4.38				4.47	4.36	4.42		
55	4.57	4.25	4.41	4.42	0.08	55	4.63	4.64	4.64	4.46	0.05
	4.31	4.62	4.47				4.38	4.32	4.35		
	4.22	4.57	4.40				4.31	4.50	4.41		
100	4.35	4.31	4.33	4.41	0.10	100	4.51	4.64	4.58	4.49	0.07
	4.53	4.48	4.51				4.50	4.46	4.48		
	4.29	4.47	4.38				4.32	4.48	4.40		
$\bar{\bar{x}}$ =4.50, NIQR=1.4, max|d|/NIQR=0.11						$\bar{\bar{x}}$ =4.42, NIQR=1.6, max|d|/NIQR=0.10					
AHD						SEM					
Day	y_{t1}	y_{t2}	$\bar{y}_t$	$\bar{\bar{y}}$	|d|	Day	y_{t1}	y_{t2}	$\bar{y}_t$	$\bar{\bar{y}}$	|d|
10	4.32	4.37	4.35	4.30	0.17	10	4.42	4.59	4.51	4.64	0.20
	4.35	4.31	4.33				4.59	4.65	4.62		
	4.21	4.25	4.23				4.87	4.74	4.81		
20	4.31	4.12	4.22	4.24	0.10	20	4.57	4.58	4.58	4.67	0.18
	4.17	4.24	4.21				4.69	4.72	4.71		
	4.27	4.31	4.29				4.75	4.68	4.72		
55	4.57	4.25	4.41	4.29	0.15	55	4.91	4.68	4.80	4.79	0.06
	4.09	4.13	4.11				4.66	4.70	4.68		
	4.36	4.32	4.34				4.91	4.86	4.89		
100	4.35	4.19	4.27	4.22	0.08	100	4.67	4.85	4.76	4.76	0.09
	4.28	4.25	4.27				4.92	4.88	4.90		
	4.16	4.08	4.12				4.64	4.58	4.61		
$\bar{\bar{x}}$ =4.13, NIQR=1.4, max|d|/NIQR=0.28						$\bar{\bar{x}}$ =4.85, NIQR=2.2, max|d|/NIQR=0.20					

Table C.14 Stability Testing Results in Sample C

Unit: μ g/kg

AOZ						AMOZ					
Day	y_{t1}	y_{t2}	$\bar{y}_t$	$\bar{\bar{y}}$	|d|	Day	y_{t1}	y_{t2}	$\bar{y}_t$	$\bar{\bar{y}}$	|d|
10	11.40	12.00	11.70	11.73	0.08	10	12.40	12.10	12.25	11.92	0.33
	12.10	11.80	11.95				11.90	11.80	11.85		
	11.60	11.50	11.55				11.70	11.60	11.65		
20	13.80	13.80	13.80	12.55	0.90	20	11.90	11.90	11.90	11.67	0.08
	12.20	12.10	12.15				11.60	11.70	11.65		
	11.80	11.60	11.70				11.40	11.50	11.45		
55	11.10	10.40	10.75	11.45	0.20	55	12.40	11.40	11.90	11.78	0.20
	11.50	11.70	11.60				11.80	12.20	12.00		
	12.10	11.90	12.00				11.50	11.40	11.45		
100	11.50	11.40	11.45	11.48	0.17	100	12.20	11.90	12.05	11.87	0.28
	11.30	11.60	11.45				11.90	11.80	11.85		
	11.70	11.40	11.55				11.70	11.70	11.70		
$\bar{\bar{x}}$ =11.65, NIQR=5.2, max|d|/NIQR=0.17						$\bar{\bar{x}}$ =11.59, NIQR=4.7, max|d|/NIQR=0.16					
AHD						SEM					
Day	y_{t1}	y_{t2}	$\bar{y}_t$	$\bar{\bar{y}}$	|d|	Day	y_{t1}	y_{t2}	$\bar{y}_t$	$\bar{\bar{y}}$	|d|
10	10.50	11.10	10.80	10.45	0.39	10	12.40	12.80	12.60	12.23	0.12
	10.40	10.20	10.30				12.10	11.90	12.00		
	10.20	10.30	10.25				12.00	12.20	12.10		
20	10.40	10.20	10.30	10.24	0.18	20	13.00	12.70	12.85	12.15	0.20
	10.20	10.70	10.45				12.20	12.00	12.10		
	9.86	10.10	9.98				11.40	11.60	11.50		
55	10.50	10.10	10.30	10.33	0.27	55	12.40	11.80	12.10	11.87	0.49
	10.50	10.30	10.40				11.50	11.70	11.60		
	10.20	10.40	10.30				12.10	11.70	11.90		
100	10.40	10.10	10.25	10.50	0.44	100	12.50	12.10	12.30	12.10	0.25
	10.70	10.60	10.65				12.20	12.40	12.30		
	10.50	10.70	10.60				11.80	11.60	11.70		
$\bar{\bar{x}}$ =10.06, NIQR=3.6, max|d|/NIQR=0.27						$\bar{\bar{x}}$ =12.35, NIQR=4.3, max|d|/NIQR=0.26					

APPENDIX D

Statistical Assessment Procedures

This appendix outlines the robust statistical assessment procedures used in this program.

D.1 Distribution of Testing Results

Since it was designed that all statistical assessments on participants' proficiency are based on the results obtained from participants, the assumption that the results follow a Normal distribution should be achieved.

Shapiro-Wilk method was used to verify the normality of the distribution of results. A statistic W for this test was calculated as follows:

$$W = \left\{\sum \alpha_K \left[x_{n+1-K} - x_K\right]\right\}^2 / \sum_{K=1}^{n}\left(x_K - \bar{x}\right)^2 \qquad \text{(D.1)}$$

where, K in the subscript is equal to 1~n/2 when n is even, or 1~(n-1)/2 when n is odd, α_K is a factor related to n and K, $\bar{x}$ is the arithmetic mean of results x. When the statistic W is larger than the critical value W(n,P) that is related to n and the confidence probability P, the testing results are normally distributed. The factors α_K and W(n,P) can be found in related statistical tables. In this program, all W values for results A, B and C of the metabolites are larger than 1.40, while the maximum critical value W(n,P) is 0.936 when n=37 and P=95%, it indicates that the results A, B and C of each metabolite follow normal distributions respectively.

Another method, kurtosis-deflection test method for normality test was used, the conclusion is consistent with that from Shapiro-Wilk method.

The histograms of z-scores for results in Appendix B.1~B.12 show approximate normal distribution as well, they were plotted by grouping and counting the numbers of z-scores with an interval of 0.5.

D.2 Summary Statistics

Robust statistics are not highly influenced by the extreme results comparing with the classical statistics that most commonly used. Instead of arithmetic mean and standard deviation, median and normalized interquartile range are used as the summary statistics for estimation of the characteristics of population, i.e. for overall performance of testing results.

Median is the middle value of a group of results, i.e. half of the results are higher than it and half are lower. It is calculated form the sorted results (from lowest to highest). If the number of results (n) is odd, the median is the singular central value. If n is even, the median is the average of the two central results. When the results were sorted as $x_{(1)} \le x_{(2)} \le \cdots \le x_{(n)}$, median is expressed in formula (D.2).

$$Median = \begin{cases} x_{(k)}, & k = (n+1)/2 \quad while \quad n \quad is \quad odd \\ [x_{(k)} + x_{(k+1)}]/2, & k = n/2 \quad while \quad n \quad is \quad even \end{cases} \tag{D.2}$$

The normalized interquartile range (NIQR) is the normalized difference between the lower and upper quartiles. The lower quartile (Q1) is the value below that a quarter of the results lie. Similarly, the upper quartile (Q3) is the value above which a quarter of the results lie. NIQR is calculated using formula (D.3). In the formula, the factor 0.7413 comes from the standard normal distribution which has a mean of zero and a standard deviation equal to one, the width of the interquartile range of such a distribution is 1.34898 and its reciprocal is 1/1.34898=0.7413.

$$NIQR = 0.7413 \times [Q(3) - Q(1)] \tag{D.3}$$

In order to express the characteristics of population, standard uncertainty of median was evaluated in this program by formula (D.4).

$$u(x) = 1.25 NIQR / \sqrt{n} \tag{D.4}$$

Details for evaluation of uncertainty refer to ISO GUM and related literatures on robust statistics.

D.3 Statistics for Assessment

A z-score is normalized value which gives a "score" to each result, relative to the other numbers in the group, a z-score value close to zero means that the result agrees well with those from the other laboratories. When robust summary statistics are used, it is a robust z-score which is calculated by formula (D.5).

$$z = \frac{x - Median}{NIQR} \tag{D.5}$$

APPENDIX E

Models of Instructions, Forms and Interim Reports

In this appendix, models of Instructions to Participants, Receipt Form and Results Sheet are copied and attached. Due to confidential requests, the parts, which include participants' information, were deleted.

INSTRUCTIONS TO PARTICIPANTS

<LabName>:

Your laboratory has been nominated by the laboratory accreditation body in your economy, <AB's name>, to participate in the Asia Pacific Laboratory Accreditation Cooperation (APLAC) Prawn Nitrofuran Metabolites Proficiency Testing Program T050. In this program, each laboratory has a unique code number. Your laboratory's code number is <LabCode>. Considering the rules of protection of participants' confidentiality, the unique code number will represent your laboratory in the reports associated with this program.

Three samples have been sent to you in aseptic bags labeled as <M_1>, <M_2>,and <M_3>. The weight of each sample is about 60 g. Enclosed with the samples are the following documents (total 13 pages):

- Instructions to Participants
- Receipt Form
- Results Sheet
- Testing Record Forms

To ensure that results from this program can be analyzed properly, you are asked to follow carefully to the instructions below.

♫ Samples were sent by mail at ambient temperature and you are requested to store them at 2-6℃ immediately upon arrival. Once the sample bags are opened, the tests must be performed immediately.

♫ You need to complete RECEIPT FORM **in English**, then **email or fax** this completed form to the coordinator **on the same day** as you receive the samples.

♫ These samples are for laboratory use only, we will take no obligation or charge arising from other purposes.

♫ Sample Description: Each sample contains ***4 kinds of metabolites of nitrofuran.*** The matrix is prawn (*Acetes Chinensis*) sauce. The names and structures of the 4 metabolites to be quantified were listed in Annex A of this file.

♫ Test method: You could analyze the samples using your preferably selected methods. It is recommended to use LC/MS or LC/MS^n methods; other suitable methods could also be used. Whatever method you selected, it should be recorded on the TESTING RECORD FORMS, and the limit of detection should be better than 0.2 μg/kg.

♫ Testing requested: The samples are for tests of ***4 metabolites of nitrofuran*** contents. According to your application, analyzing part or all kinds of the residues is permitted, but all samples must be

analyzed and reported. It is recommended that each sample should be mixed, prior to test, thoroughly and respectively, and the mass of each duplicated test portion should exceed 2.0 g. Please record relevant information on TESTING RECORD FORMS in details, and all you report will be benefit for analyzing testing results.

♫ Reporting results: For each residue in each sample, please report only one mean result respectively on the RESULT SHEET in corresponding structures listed in Annex A, with the number of duplicated tests from which the mean result was obtained. The test results shall be corrected by recovery. Measurement uncertainty shall be evaluated and reported referring to Annex B for each result. In this program, z-scores will be used as the main method for assessing the performance of participants, no influence will be with reporting uncertainty.

Please fill out all relevant information, then email or fax the RESULT SHEET to the coordinator of this program and your accreditation body **NO LATER THAN THE DEADLINE 20 March. 2008**. And the TESTING RECORD FORMS, relevant spectrums, your preferred form of Measurement Uncertainty Report and any supporting documents should be submitted to your accreditation body before the deadline. At the same time, email or mail all the documents to:

Coordinator

Mr. Zheng Jiang or Ms. Xiao Shanshan

Technology Center, Liaoning Entry-Exit Inspection & Quarantine Bureau of P. R. China

39, Renmin Road, Dalian, 116001, P.R. China

Phone/Fax: +86-0411-8258 3822/8258 3674

Email: PTC_China@126.com

Mr.Cao Zhijun

CNAS, The People's Republic of China

Annex A

（for Instruction to Participants）

Structures of Veterinary Drugs to Be Analyzed

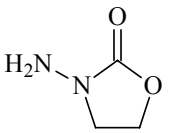
AOZ:

3-amino-2-oxazolidone

Molecular Formula: $C_3H_6N_2O_2$,

CAS Registering Number: 80-65-9

Structure:



AOZ（$C_3H_6N_2O_2$）

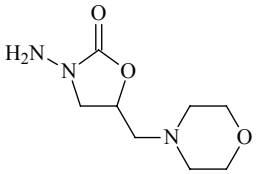
AMOZ:

3-amino-5-morpholinomethyl-2-oxazolidone

Molecular Formula: $C_8H_{15}N_3O_3$

CAS Registering Number: 43056-63-9

Structure:



AMOZ ($C_8H_{15}N_3O_3$)

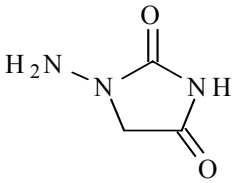
AHD:

1-amino-hydantoin

Molecular Formula: $C_3H_5N_3O_2$,

CAS Registering Number: $C_3H_5N_3O_2$·HCL, 2827-56-7

Structure:



AHD（$C_3H_5N_3O_2$）

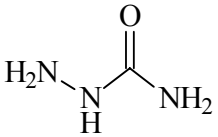
SEM:

semicarbazide

Molecular Formula: CH_5N_3O

CAS Registering Number: 57-56-7

Structure:



SEM (CH_5N_3O)

Annex B

（for Instruction to Participants）

Instructions for Quantifying Measurement Uncertainty

Part 1 and Part 2 of this Annex are reproduced from APLAC PT002-2003.

Part 1 Background information & justification for this change

ISO/IEC 17025[1] requires that, except under specified conditions, the uncertainty of measurement associated with the results of tests and measurements must be estimated.

What is uncertainty of measurement?

Uncertainty of measurement is defined as a "parameter, associated with the result of a measurement, that characterizes the dispersion of the values that could reasonably be attributed to the measurand" (the measurand is the particular quantity subject to measurement).

The result of a test or measurement is our best estimate of the true value of the measurand. The result is imperfect. The true value of the measurand is contained within a range of values about the measurement result and the "uncertainty of measurement" is an estimate of the magnitude of that range expressed at a given level of confidence (confidence interval). Uncertainty of measurement is usually given a 95% confidence interval and would normally be expressed in the appropriate SI units (ie. mm, ℃, g/l, Mpa etc).

For example, the result of a measurement might be 5.1 mg/l with an uncertainty of ±0.2mg/l at a 95% level of confidence. This means that there is an estimated 95% probability that the true value is in the range 4.9mg/l to 5.2mg/l. The 95% probability means that there is an estimated one in twenty chance that the true value is outside that range.

Uncertainty of measurement may also be expressed as a percentage where appropriate.

To assist laboratories to comply with the requirements of ISO/IEC 17025 for estimating uncertainty and to promote a uniform methodology in this estimation, information packages for APLAC PT testing program participants now include general guidance relating to estimating uncertainties for the specific testing involved, and final program reports will now include relevant worked examples. All program participants are required to report their estimates of uncertainty to their accreditation bodies along with their results unless the Technical Adviser to the program specifically waives any requirement to estimate uncertainties. The estimates of uncertainty provided by participants will be incorporated into the final program reports enabling direct comparison of uncertainty estimates across the program participants. The uncertainty estimates will not be used in the evaluation of the results on the primary samples.

How is uncertainty of measurement to be estimated?

APLAC expects that program participants' uncertainties of measurement would be estimated in

accordance with the requirements of the respective member accreditation bodies. There are different approaches and methodologies available. Worked examples provided in APLAC PT program reports will generally be based on ISO GUM but will recognize other methodologies in accordance with 5.4.6.3 Note 3 in ISO 17025.

Estimates of uncertainty of measurement provided by program participants are required to be given at the 95% level of confidence.

ISO GUM methodology

An estimate of uncertainty of measurement would usually be based on the combination of a number of influencing parameters (components of uncertainty) such as errors in reference values, instrument errors, repeatability, thermal effects, weighing errors, inhomogeneity etc. ISO GUM methodology requires that the influence of each component of uncertainty on the measurement result be quantified and expressed numerically as a standard deviation. These values are then combined according to the rules of the propagation of uncertainty to produce a combined standard deviation (combined standard uncertainty) and the combined standard uncertainty is multiplied by a coverage factor to produce an expanded uncertainty at the required level of confidence. Detailed descriptions and information on the implementation of this methodology have been published by ISO2, UKAS3 and Eurachem/CITAC4 and made available over the internet7.

Uncertainty of measurement is best estimated within the individual laboratory environment. All factors which will have a significant influence on the test or measurement result must be included in the estimation process. There must be suitable programs utilising reference standards, instruments and materials to ensure on-going and adequate quality control and repeatability and reproducibility of methods and equipment over time. In many instances, it will be possible to utilise quality control data in assessing uncertainty components such as precision. Where these data are not available, it may be necessary to carry out precision studies or to rely on published information about the method or portions of it until the laboratory can obtain its own estimates.

APLAC is aware of the general need for better estimates of uncertainty, and estimates that are obtained under similar conditions in all laboratories. PT programs are useful mechanisms for spreading awareness of uncertainty of measurement and the effects of different ways of estimating it. We anticipate that the information made available through PT programs will help focus discussions on uncertainty of measurement.

APLAC Technical Committees will interpret the information and report on current practices. They will also make recommendations for improving the collection of uncertainty data, the estimation of uncertainties and incorporating data and information on uncertainty of measurement into PT program reports. Therefore we anticipate an evolution in the mechanisms for collecting and reporting uncertainty data and associated information over the next few years.

Participation in APLAC PT programs should assist laboratories to develop appropriate estimates of uncertainty, help to guide accreditation bodies to adopting common and consistent approaches leading to enhanced understanding and international comparability of measurements among the

member nations.

APLAC will consider the use of estimates of uncertainty of measurement in the evaluation of its PT testing program results after is satisfied that participating laboratories are estimating uncertainties of measurement in an appropriate and consistent manner.

References

1. ISO/IEC 17025:2005, General requirements for the competence of testing and calibration laboratories. ISO, Geneva(2005).
2. Guide to the Expression of Uncertainty in Measurement. ISO, Geneva(1993)
3. UKAS LAB 12: The Expression of Uncertainty in Testing. UKAS, London(2000)
4. Eurachem/CITAC Guide QUAM:2000, P1. Quantifying Uncertainty in Analytical Measurement, 2nd Edition (2000)
5. ISO 21748:2004, Guide for the use of repeatability, reproducibility, and trueness estimates in measurement uncertainty estimation.
6. APLAC – Policy, interpretation and guidance on the estimation of uncertainty of measurement in testing (Draft 5 September 2002)
7. Websites:
8. www.A2LA.org/ (for A2LA policies, links to guidance documents, including the UKAS Guide, and the Eurachem/CITAC Guide, at no cost)
9. www.measurementuncertainty.org/ (Eurachem/CITAC Guide)
10. www.fasor.com/iso25/ (general information, links, and discussion of ISO-IEC 17025)

Part 2 Evaluating participants' results

All APLAC PT program result sheets will provide a column on the PT results sheet to report an estimate of uncertainty of measurement for each reported measurement result. All estimates of uncertainty of measurement must be given as a 95% confidence interval ($k \approx 2$). The estimates would then appear in the final report, along with any relevant interpretations from experts. Detailed information on the estimation of the reported uncertainties must be provided to the relevant accreditation bodies for review and assessment.

The objective is to inform the participants so they can review and refine their own procedures and methodologies.

Program providers and technical advisers will include the reported uncertainty estimates either as summary data or directly with the submitted results.

APLAC PT testing programs will continue to use z-scores as the primary method for evaluating the relative performance of individual participant laboratories until such time as it is appropriate to incorporate quoted uncertainty estimates into the evaluation process and to assess performance using the En ratio method.

Part 3 Cases in this program

Considering the situation and needs available on evaluating uncertainty in this program, especially

some parameters could not be obtained from the participants, this part of Annex B outlines the information of these factors as follows.

Heterogeneity and Instability of the Samples The samples are stable during at least 45 days at ambient temperature in dark condition, the uncertainty caused by instability of the samples sent to you are far less than some main components of uncertainty such as the uncertainty caused by extraction and analysis processes. As well, the samples are homogeneous enough, the uncertainty of heterogeneity could be ignored. Another case is that the uncertainty of heterogeneity and instability may be included in other components when some special evaluation methods are used.

Precision The precision may vary depending on the testing methods, various approaches are available for estimating the repeatability and/or reproducibility. When Type A evaluation is employed for evaluating repeatability, special caution should be paid on testing processes due to limited masses of the samples.

Level of Confidence In this program, if the level of confidence other than 95% (or k=2) intends to be used, the estimates of uncertainty with relevant information, such as the actual level of confidence, degrees of freedom or coverage factor, shall be reported in your Measurement Uncertainty Report in a proper form listed in ISO GUM.

Annex C

（for Instruction to Participants）

A Commonly Used Method for Preparation and Storing up the Reference Materials and Their Solutions

Participating laboratory should pay attention to that ***nitrofuran metabolites*** are unstable in light. During handling and storage, it is suggested that suitable method should be used for keeping ***nitrofuran metabolites*** reference materials and their stock solutions away from light.

Additionally, when detected by LC/MC or LC/MS^n, ***nitrofuran metabolites*** have different response values in different media. It is suggested that ***nitrofuran metabolites*** reference materials should be dissolved firstly by methanol or water of the right weight, and further diluted to a desired concentration with the mobile phase, that is, the medium of the working solutions used for LC/MS analysis should be the mobile phase.

Annex D

（for Instruction to Participants）

Recommended Pretreatment Protocol

Each prawn sauce sample was directly extracted and purified, then the eluate could be analyzed by LC/MS or LC/MS^n.

Extraction Solution: ethyl acetate

Mobile Phase: acetonitrile+0.1% formic acid, gradient elution

Procedures

- Weigh 5.0g of the prawn sauce in 50 mL plastic centrifuge tube.
- Add suitable internal standard solution and 2,4-dinitro-benzaldehyde solution, then add 10 mL 0.2 mol/L HCl. After mixed about 2 minutes, the tube is keep in dark for 16 h at 37℃.
- After adjusting the pH of the solution to 7.0~8.0 by 1.0 mol/L NaOH, add extraction solution to extract the sample for several times. Collect all extracted solution.
- Evaporate the extracted solution to dryness at 45℃.
- Add 1 mL mobile phase to dissolve the residues thoroughly.
- Purify eluate with n-hexane, then the layer of n-hexane is discarded and the clear layer left, filtered through 0.45 μm membrane filter, and then the purified eluate is used for LC/MS analysis.

RECEIPT FORM

We are pleased to announce that in accordance with the distribution schedule of APLAC Prawn Nitrofuran Metabolites Proficiency Testing Program T050, we have dispatched the samples on 20 Jan. 2008 through TNT Express, along with Instructions to Participants, Receipt Form, Result Sheet and Testing Record Forms.

In order to monitor status of the samples, we kindly ask each participating laboratory on receipt of the samples to fill out this RECEIPT FORM and return a copy by **email or fax URGENTLY to**:

Mr. Zheng Jiang or Ms. Xiao Shanshan

Fax: +86-411-82583674

Email: PTC_China@126.com

Thank you in advance for your cooperation.

NAME OF LABORATORY: <LabName>

LAB CODE: <LabCode>

DATE SAMPLE RECEIVED:_____ SAMPLE AMOUNT:_____

The samples are labeled as follows: <M_1>, <M_2>, <M_3>

Inspect the packaging. Was there any visible damage? ☐ **Yes** ☐ **No**

If yes, or any other problem is available, describe the problem below.

_____

_____

_____

_____

Signature: **Date:**

Results Sheet

Lab Code: <LabCode>

SAMPLE RECEIVED DATE: _____ REPORT DATE: _____
SAMPLE PROCESSED DATE: From_____to_____

Sample	**Testing Result (μg/kg) of AOZ** With Measurement Uncertainty	**Number of Duplicate tests**	**Limit of Detection (μg/kg)**
<M_1>			
<M_2>			
<M_3>			
Testing Method			
Sample	**Testing Result (μg/kg) of AMOZ With Measurement Uncertainty**	**Number of Duplicate tests**	**Limit of Detection (μg/kg)**
<M_1>			
<M_2>			
<M_3>			
Testing Method			
Sample	**Testing Result (μg/kg) of AHD With Measurement Uncertainty**	**Number of Duplicate tests**	**Limit of Detection (μg/kg)**
<M_1>			
<M_2>			
<M_3>			
Testing Method			
Sample	**Testing Result (μg/kg) of SEM With Measurement Uncertainty**	**Number of Duplicate tests**	**Limit of Detection (μg/kg)**
<M_1>			
<M_2>			
<M_3>			
Testing Method			

(Attention: the concentration of AHD should be calculated by AHD instead of AHD·HCL)

Signature: Date:

Return this Results Sheet by **no later than the deadline 20 March. 2008** by fax or email, and then mail to:

Mr. Zheng Jiang or Ms. Xiao Shanshan
Technology Center, Liaoning Entry-Exit Inspection & Quarantine Bureau of P. R. China
39, Renmin Road, Dalian, 116001, P.R. China
Phone: +86-411-82583822, Fax: +86-411-82583674, Email: PTC_China@126.com

Testing Record Forms

Lab Code:<LabCode>

Is the method used for the results submitted in your scope of accreditation?			☐ yes	☐ no
Reference:	☐ standard/ ☐ journal/ ☐ book	year	No./Vol /Edition	pages
Determination method	☐ LC/MS ☐ LC/MS^n ☐ HPLC ☐ Other method _____			
Protocol for Quantification	☐ External Standards ☐ Internal Standards ☐ Others (specify): _____			
Reference Materials	Names or Structures: Supplier(s)： Purity：			
Reagents and Materials				
Extraction	Extraction Method: Instruments used:			
Cleanup	Cleanup Method: Instruments used:			
Concentration	Concentration Method: Instruments used:			
LC/MS Operating	Equipment Model：_____Ion Source:_____			

Condition	Column (type and specification):_____ Mobile Phase:_____Flow Speed:_____ mL/min Injection Volume：_____μL Column Temperature:_____ ℃
Testing Sample	Mass of Test Portions (in g) <M_1>: From_____to_____ <M_2> : From_____to_____ <M_3> : From_____to_____
Chromatogram	Chromatograms Attached: _____Pages The retention time of ***AOZ*** is _____min. The retention time of ***AMOZ*** is _____min. The retention time of ***AHD*** is _____min. The retention time of ***SEM*** is _____min.
Calculating Formulae	
Additional Information	**Method usually used for Extraction (including instruments used):** **11** Solvent extraction **11** Immerging method **11** Shaking method **11** Homogenization **11** Soxhlet extraction **11** SFE **11** Other (specify):_____ **Method usually used for Purification (including instruments used):** **11** L-L partition **11** Column adsorption **11** SPE **11** GPC **11** SFE **11** Other (specify):_____ **Method usually used for Concentration (including instruments used):** **11** Speedy centrifugation **11** On-line concentration **11** Rotary evaporation **11** N_2 blowing instrument **11** natural evaporation **11** K.D concentration instrument **11** Other (specify):_____ (Extra sheet may be added if necessary)

Signature： **Date:**

Asia Pacific Laboratory Accreditation Cooperation

APLAC Prawn Nitrofuran Metabolites Proficiency Testing Program T050

INTERIM REPORT

Date of Issue : April 29, 2008

In this APLAC proficiency testing program, the contents of nitrofuran metabolites, AOZ, AMOZ, AHD and SEM in prawn sauce samples were tested.
Three gross samples A, B and C were prepared and divided respectively to testing samples A, B and C, then every three testing samples A, B and C were distributed to the participating laboratories.
The results for the tests on content of AOZ, AMOZ, AHD and SEM reported from the participating laboratories are listed in Table 1, 3, 5 and 7. Results reported on Sample A, B and C are listed in corresponding column Result A, Result B and Result C, respectively.
The statistics given in Table 2, 4, 6 and 8 were calculated by robust statistical methods based on the results listed in Table 1, 3, 5 and 7, respectively. The z-scores can be calculated by the following formula. In the Formula, Result is an individual Result A, Result B or Result C, respectively.

Formula: z-score=(Result- Median)/ NIQR

As a general rule, any z-score outside the range of -2 to 2 indicates a questionable result/pair of results, while an outlier is any result/pair of results with a z-score outside the range of -3 to 3.
Please confirm this interim report NO LATER THAN MAY 18, 2008 with your lab's address, Lab Code No. in this program, email, phone and fax number, we will use them for sending the final report. Thanks for your cooperation.
If you have any question on any aspect of your test, or on this PT program, please feel free to contact the persons listed below.

PT Manager:

Mr. Cao Zhijun
CNAS, China
Web: www.cnas.org.cn
E-mail: caozj@cnas.org.cn

Coordinators:

Mr. Lin Weixuan
Fax: +86 411 82583674
Phone: +86 411 82583822
E-mail: PTC_China@126.com

Mr. Zheng Jiang

Dr. Xiao Shanshan

CNAS PROFICIENCY TESTING CNAS PT0005

Table 1 Reported Results of Contents of AOZ

LabCode	Sample A	Result A (μg/kg)	Sample B	Result B (μg/kg)	Sample C	Result C (μg/kg)	Testing Method	LOD (μg/kg)
T050-001	M051	3.8±0.2	M001	6.7±0.2	M098	11.7±0.2	LC/MS/MS	0.1
T050-002	M054	3.7±0.2	M003	8.4±0.2	M102	12.5±0.2	LC/MS/MS	0.1
T050-003	M055	2.28±0.10	M005	5.72±0.44	M103	5.88±0.98	LC/MSn	0.06
T050-004	M060	2.20	M012	4.32	M108	5.09	LC/MSn	0.16
T050-005	M063	4.5±0.5	M013	6.8±0.7	M109	11.5±1.0	LCMS	1
T050-006	M064	4.26±0.54	M017	7.34±0.94	M115	12.08±1.55	LC/MSn	0.109
T050-007	M067	5.44 ± 0.51	M023	8.27 ± 0.51	M117	16.4 ± 0.51	LC/MS/MS	0.3
T050-008	M070	4.808±0.235	M024	6.453±0.235	M072	8.862±0.235	ELISA	0.6
T050-010	M076	4.13±0.45	M030	7.82±0.61	M077	11.80±2.68	ELISA	0.2
T050-011	M081	5.03±0.66	M033	9.04±2.06	M079	12.34±1.42	ELISA	0.150
T050-012	M082	6.5±0.5	M035	11.8±1	M084	20.2±2	LC/MSn	0.1
T050-013	M086	3.38±0.28	M037	11.6±0.95	M085	18.4±1.51	LC/MSn	0.90
T050-014	M092	5.7±0.3	M040	9.2±0.4	M088	15.4±0.7	LC/MS/MS	0.2
T050-015	M095	6.2±1.81	M044	11.1±3.22	M090	18.8±5.44	LC/MS/MS	0.29
T050-016	M096	4.21±0.48	M048	7.51±1.00	M093	9.56±0.84	LC/MS/MS	0.2
T050-017	M002	1.8±0.9	M050	2.9±1.5	M004	5.0±2.5	LC/MSn	0.1
T050-018	M006	5±1	M052	7±2	M007	12±3	LC/MS/MS	1
T050-019	M008	3.08	M056	4.10	M009	5.97	LC/MSn	1
T050-020	M011	4.97±0.60	M058	9.01±0.47	M010	13.83±0.94	LCMS	0.1
T050-021	M014	4.861±0.778	M061	8.033±1.285	M015	10.402±1.664	LC/MSn	0.092
T050-022	M018	4.06	M065	7.32	M016	12.53	LC/MSn	0.01
T050-023	M020	7.41	M069	12.3	M019	20.6	LC/MS/MS	0.2
T050-024	M022	4.0±0.80	M071	7.6±1.5	M021	12.6±2.5	LC/MSn	1.0
T050-025	M026	1.3±0.07	M074	2.3±0.12	M027	2.8±0.14	LC/MSn	<0.2
T050-026	M028	4.9	M078	7.5	M029	12.0	LC/MS/MS	0.5
T050-027	M031	5.0±11%	M080	10.9±11%	M032	8.7±11%	LC/MS/MS	0.1
T050-028	M034	2.21±0.25	M083	3.76±0.25	M036	6.01±0.25	LC/MSn	0.15
T050-029	M039	3.7±20%	M087	5.8±20%	M038	7.2±20%	LC/MSn	0.5
T050-030	M042	2.77	M089	4.30	M041	5.89		0.07
T050-031	M045	5.50±0.15	M091	8.07±0.22	M043	8.31±0.22	ELISA	0.2
T050-032	M047	1.4±0.14	M094	2.7±0.27	M046	4.4±0.44		0.02
T050-033	M120	0.23±21%	M119	0.51±21%	M049	0.84±21%	LC/MSn	0.1
T050-034	M101	2.80	M100	2.74	M053	0.62	LC/MSn	0.021
T050-035	M105	4,74 ± 1,03	M104	11,76 ± 2,56	M057	26,72 ± 5,81	UPLC/MS/MS	0.16
T050-037	M110	5±1	M111	8±1	M062	11±2	LC/MS/MS	1
T050-038	M112	5.58±0.64	M113	9.17±0.66	M066	15.2±0.99	LC/MSn	0.10
T050-039	M116	4.5±1.6	M114	7.4±2.7	M068	13±4.7		0.2

Table 2 Statistics for the Test on Contents of AOZ

	Total Number of Results	Median, μg/kg	NIQR, μg/kg
Result A	37	4.3±0.6, k=2	1.4
Result B	37	7.5±1.0, k=2	2.4
Result C	37	11.7±2.1, k=2	5.2

Table 3 Reported Results of Contents of AMOZ

LabCode	Sample A	Result A (μ g/kg)	Sample B	Result B (μ g/kg)	Sample C	Result C (μ g/kg)	Testing Method	LOD (μ g/kg)
T050-001	M051	2.4±0.2	M001	5.6±0.2	M098	9.2±0.2	LC/MS/MS	0.1
T050-002	M054	3.8±0.2	M003	7.9±0.2	M102	12.4±0.2	LC/MS/MS	0.1
T050-003	M055	3.79±0.20	M005	8.06±0.67	M103	8.37±0.44	LC/MSn	0.06
T050-004	M060	2.18	M012	4.48	M108	5.40	LC/MSn	0.16
T050-005	M063	4.3±0.4	M013	6.8±0.7	M109	10.8±1.0	LC/MS	1
T050-006	M064	4.65±0.52	M017	8.09±0.90	M115	13.80±1.53	LC/MSn	0.091
T050-007	M067	8.18 ± 0.49	M023	11.7 ± 0.49	M117	16.03 ± 0.49	LC/MS/MS	0.3
T050-008	M070	4.207±0.232	M024	7.500±0.232	M072	9.691±0.232	ELISA	0.8
T050-010	M076	3.58±0.30	M030	5.73±1.49	M077	10.64±2.60	ELISA	0.2
T050-011	M081	4.12±0.92	M033	9.39±1.42	M079	13.65±1.72	ELISA	0.300
T050-012	M082	0.5±0.05	M035	4.6±0.5	M084	11.7±1	LC/MSn	0.1
T050-013	M086	4.41±0.27	M037	7.61±0.47	M085	11.3±0.70	LC/MSn	0.42
T050-014	M092	5.4±0.3	M040	9.2±0.5	M088	15.0±0.8	LC/MS/MS	0.2
T050-015	M095	9.1±1.67	M044	16.1±2.98	M090	27.0±5.02	LC/MS/MS	0.02
T050-016	M096	5.30±0.80	M048	8.96±2.00	M093	11.7±2.22	LC/MS/MS	0.2
T050-017	M002	4.8±2.4	M050	7.5±3.8	M004	9.8±3.1	LC/MSn	0.5
T050-018	M006	5±1	M052	9±1	M007	16±2	LC/MS/MS	1
T050-019	M008	3.83	M056	4.18	M009	4.65	LC/MSn	1
T050-020	M011	5.59±0.27	M058	8.55±0.17	M010	15.38±0.49	LC/MS	0.1
T050-021	M014	4.470±0.939	M061	7.450±1.564	M015	9.572±2.010	LC/MSn	0.159
T050-022	M018	4.85	M065	7.88	M016	15.24	LC/MSn	0.01
T050-023	M020	6.33	M069	9.32	M019	14.8	LC/MS/MS	0.2
T050-024	M022	5.6±1.1	M071	8.6±1.7	M021	15.1±3.0	LC/MSn	1.0
T050-025	M026	2.1±0.11	M074	3.5±0.18	M027	4.3±0.22	LC/MSn	<0.2
T050-026	M028	6.8	M078	9.3	M029	13.4	LC/MS/MS	0.5
T050-027	M031	6.85±13%	M080	11.4±13%	M032	12.6±13%	LC/MS/MS	0.1
T050-028	M034	1.44±0.25	M083	2.25±0.25	M036	4.14±0.25	LC/MSn	0.19
T050-029	M039	4.3±20%	M087	7.4±20%	M038	8.5±20%	LC/MSn	0.5
T050-030	M042	1.78	M089	2.95	M041	3.97		0.18
T050-032	M047	1.2±0.1	M094	2.2±0.18	M046	3.6±0.29		0.02
T050-033	M120	0.21±19%	M119	0.46±19%	M049	0.73±19%	LC/MSn	0.1
T050-034	M101	1.64	M100	1.60	M053	0.79	LC/MSn	0.027
T050-035	M105	7,97 ± 0,81	M104	11,01 ± 1,12	M057	29,72 ± 3,01	UPLC/MS/MS	0.05
T050-037	M110	5±1	M111	8±2	M062	11±2	LC/MS/MS	1
T050-038	M112	5.61±0.25	M113	9.48±0.54	M066	16.1±0.56	LC/MSn	0.10
T050-039	M116	5.2±1.4	M114	8.6±2.2	M068	14±3.6		0.2

Table 4 Statistics for the Test on Contents of AMOZ

	Total Number of Results	Median, μ g/kg	NIQR, μ g/kg
Result A	36	4.4±0.7, k=2	1.6
Result B	36	7.9±1.1, k=2	2.7
Result C	36	11.5±2.0, k=2	4.7

Table 5 Reported Results of Contents of AHD

LabCode	Sample A	Result A (μg/kg)	Sample B	Result B (μg/kg)	Sample C	Result C (μg/kg)	Testing Method	LOD (μg/kg)
T050-001	M051	2.4±0.2	M001	4.5±0.2	M098	7.9±0.2	LC/MS/MS	0.3
T050-002	M054	4.5±0.2	M003	8.8±0.2	M102	14.1±0.2	LC/MS/MS	0.2
T050-003	M055	3.41±0.18	M005	9.70±1.35	M103	8.62±0.77	LC/MSn	0.10
T050-004	M060	2.62	M012	4.97	M108	5.63	LC/MSn	0.48
T050-005	M063	4.1±0.4	M013	5.6±0.5	M109	10.2±1.0	LC/MS	1
T050-006	M064	5.71±0.72	M017	9.15±1.15	M115	19.64±2.47	LC/MSn	0.115
T050-007	M067	4.90 ± 0.5	M023	8.40 ± 0.5	M117	15.8 ± 0.5	LC/MS/MS	0.3
T050-012	M082	3.5±0.3	M035	7.1±0.5	M084	11.4±1	LC/MSn	0.1
T050-013	M086	3.68±0.77	M037	5.72±1.20	M085	9.54±2.00	LC/MSn	0.96
T050-014	M092	3.3±0.4	M040	6.3±0.7	M088	9.5±1.0	LC/MS/MS	0.2
T050-015	M095	4.6±1.38	M044	8.6±2.54	M090	14.0±4.18	LC/MS/MS	0.01
T050-016	M096	5.92±0.31	M048	8.96±1.56	M093	13.2±1.31	LC/MS/MS	0.2
T050-017	M002	7.0±3.5	M050	7.2±3.6	M004	13.3±4.3	LC/MSn	5
T050-018	M006	4±1	M052	8±2	M007	13±2	LC/MS/MS	1
T050-019	M008	1.84	M056	2.20	M009	3.41	LC/MSn	1
T050-020	M011	4.39±0.72	M058	8.63±0.75	M010	12.13±2.01	LC/MS	0.1
T050-021	M014	5.263±0.947	M061	8.808±1.585	M015	11.353±2.043	LC/MSn	0.204
T050-022	M018	4.19	M065	8.53	M016	13.57	LC/MSn	0.01
T050-023	M020	4.41	M069	6.55	M019	12.0	LC/MS/MS	0.2
T050-024	M022	4.5±0.9	M071	7.4±1.5	M021	12.2±2.4	LC/MSn	1.0
T050-025	M026	2.7±0.14	M074	4.6±0.23	M027	5.5±0.28	LC/MSn	<0.2
T050-026	M028	4.9	M078	7.5	M029	11.7	LC/MS/MS	0.5
T050-027	M031	5.7±14%	M080	8.0±14%	M032	9.6±14%	LC/MS/MS	0.5
T050-028	M034	2.53±0.25	M083	4.69±0.25	M036	7.46±0.25	LC/MSn	NA
T050-029	M039	1.8±20%	M087	2.5±20%	M038	3.1±20%	LC/MSn	
T050-032	M047	3.2±0.45	M094	6.5±0.91	M046	9.5±1.33		0.05
T050-033	M120	0.21±23%	M119	0.49±23%	M049	0.76±23%	LC/MSn	0.1
T050-034	M101	0.70	M100	2.74	M053	1.07	LC/MSn	0.085
T050-037	M110	6±1	M111	9±2	M062	11±2	LC/MS/MS	1
T050-038	M112	5.02±0.34	M113	8.24±0.56	M066	14.5±0.70	LC/MSn	0.20
T050-039	M116	3.8±1.6	M114	6.3±2.7	M068	11±4.7		0.2

Table 6 Statistics for the Test on Contents of AHD

	Total Number of Results	Median, μg/kg	NIQR, μg/kg
Result A	31	4.1±0.6, k=2	1.4
Result B	31	7.2±1.1, k=2	2.4
Result C	31	11.0±1.6, k=2	3.6

Table 7 Reported Results of Contents of SEM

LabCode	Sample A	Result A (μg/kg)	Sample B	Result B (μg/kg)	Sample C	Result C (μg/kg)	Testing Method	LOD (μg/kg)
T050-001	M051	2.9±0.2	M001	5.6±0.2	M098	9.0±0.2	LC/MS/MS	0.5
T050-002	M054	6.3±0.2	M003	11.3±0.2	M102	16.7±0.2	LC/MS/MS	0.2
T050-003	M055	7.52±0.47	M005	12.36±1.48	M103	15.17±1.37	LC/MSn	0.19
T050-004	M060	2.39	M012	4.66	M108	5.53	LC/MSn	0.38
T050-005	M063	5.0±0.5	M013	16.0±1.6	M109	10.8±1.0	LC/MS	1
T050-006	M064	4.02±0.45	M017	6.53±0.73	M115	12.54±1.40	LC/MSn	0.159
T050-007	M067	6.44 ± 0.48	M023	12.6 ± 0.48	M117	19.46 ± 0.48	LC/MS/MS	0.3
T050-012	M082	6.7±0.5	M035	12.1±1	M084	20.0±2	LC/MSn	0.1
T050-013	M086	9.27±0.66	M037	14.2±1.02	M085	23.7±1.68	LC/MSn	0.51
T050-014	M092	3.3±0.5	M040	6.3±0.9	M088	9.0±1.3	LC/MS/MS	0.2
T050-015	M095	5.1±0.87	M044	8.9±1.52	M090	15.0±2.58	LC/MS/MS	0.10
T050-016	M096	5.32±0.55	M048	8.94±1.84	M093	14.4±1.60	LC/MS/MS	0.2
T050-017	M002	5.9±3.0	M050	8.3±4.2	M004	15.6±5.0	LC/MSn	0.1
T050-018	M006	5±1	M052	8±2	M007	13±3	LC/MS/MS	1
T050-019	M008	1.88	M056	3.26	M009	2.64	LC/MSn	1
T050-020	M011	2.75±0.29	M058	5.73±0.33	M010	7.85±0.86	LC/MS	0.1
T050-021	M014	4.449±0.801	M061	7.148±1.267	M015	9.251±1.665	LC/MSn	0.074
T050-022	M018	4.18	M065	7.11	M016	11.01	LC/MSn	0.01
T050-023	M020	5.04	M069	7.64	M019	14.2	LC/MS/MS	0.2
T050-024	M022	12.8±2.6	M071	17.1±3.4	M021	19.0±3.8	LC/MSn	1.0
T050-025	M026	2.2±0.11	M074	4.0±0.20	M027	4.4±0.22	LC/MSn	<0.2
T050-026	M028	5.2	M078	7.2	M029	10.4	LC/MS/MS	0.5
T050-027	M031	6.5±16%	M080	10.0±16%	M032	10.7±16%	LC/MS/MS	0.5
T050-028	M034	2.95±0.25	M083	3.24±0.25	M036	7.92±0.25	LC/MSn	NA
T050-029	M039	6.6±20%	M087	12.2±20%	M038	12.9±20%	LC/MSn	0.5
T050-032	M047	4.3±0.52	M094	8.2±0.98	M046	13.7±1.64		0.05
T050-033	M120	0.60±32%	M119	1.39±32%	M049	1.94±32%	LC/MSn	0.4
T050-034	M101	1.72	M100	1.08	M053	2.22	LC/MSn	0.097
T050-037	M110	5±1	M111	7±2	M062	10±3	LC/MS/MS	1
T050-038	M112	5.18±0.18	M113	8.23±0.24	M066	14.5±0.68	LC/MSn	0.20
T050-039	M116	4.7±3.0	M114	7.2±4.6	M068	13±8.3		0.4

Table 8 Statistics for the Test on Contents of SEM

	Total Number of Results	Median, μg/kg	NIQR, μg/kg
Result A	31	5.0±1.0, k=2	2.2
Result B	31	7.6±1.5, k=2	3.4
Result C	31	12.5±1.9, k=2	4.3