CERTIFICATION

AOAC Research Institute

Performance Tested Methods[SM]

Certificate No.

011804

The AOAC Research Institute hereby certifies the method known as:

Wheat/Gluten ELISA Kit

manufactured by

Morinaga Institute of Biological Science, Inc.
SAHIURA 2-1-16
KANAZAWA-KU
YOKOHAMA-SHI
236-0003, Japan

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Scott Coates

Scott Coates, Senior Director
Signature for AOAC Research Institute

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METHOD NAME
Wheat/Gluten ELISA Kit

CATALOG NUMBER
M2103

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APPLICABILITY OF METHOD
Target analyte – wheat protein/gluten (from wheat, barley, and rye cereals).

Matrixes – (1.0 g or 1mL): ice cream, water, soup, cider, rice flour, gluten-free bread

Performance claims - Mean recovery and precision

I. **Overnight extraction and low range assay: 83 ± 3.4 % for water spiked with gliadin, 79 ± 2.3 % for ice cream spiked with gliadin, 88 ± 0.8 % for soup spiked with gliadin, 110 ± 6.4% for water spiked with wheat flour, 108 ± 10.3 % for cider spiked with wheat flour, and 121 ± 6.4% for rice flour.**
II. **Short time extraction and low range assay: 83 ± 1.9 % for water spiked with gliadin, 80 ± 2.8 % for ice cream spiked with gliadin, 84 ± 2.1 % for soup spiked with gliadin, 111 ± 10.8% for water spiked with wheat flour, 107 ± 9.2 % for cider spiked with wheat flour, and 130 ± 11.2% for rice flour.**
III. **Overnight extraction and low range assay: 86 ± 2.0 % for water spiked with gliadin, 85± 1.2 % for ice cream spiked with gliadin, 88 ± 1.7 % for soup spiked with gliadin, 119 ± 4.2% for water spiked with wheat flour, 121 ± 1.8 % for cider spiked with wheat flour, and 130 ± 4.3% for rice flour.**
IV. **Short time extraction and high range assay: 86 ± 1.2 % for water spiked with gliadin, 85 ± 1.4 % for ice cream spiked with gliadin, 88 ± 1.7 % for soup spiked with gliadin, 122 ± 3.5% for water spiked with wheat flour, 121 ± 4.0 % for cider spiked with wheat flour, and 141 ± 2.7% for rice flour.**

Mean limit of detection:

I. **Overnight extraction and low range assay: 0.08 ppm for water, 0.04 ppm for ice cream, 0.07 ppm for soup, 0.03 ppm for cider, and 0.05 ppm for rice flour.**
II. **Short time extraction and low range assay: 0.05 ppm for water, 0.03 ppm for ice cream, 0.05 ppm for soup, 0.02 ppm for cider, and 0.07 ppm for rice flour.**
III. **Overnight extraction and high range assay: 0.03 ppm for water, 0.03 ppm for ice cream, 0.15 ppm for soup, 0.16 ppm for cider, and 0.14 ppm for rice flour.**
IV. **Short time extraction and high range assay: 0.13 ppm for water, 0.05 ppm for ice cream, 0.15 ppm for soup, 0.10 ppm for cider, and, 0.07 ppm for rice flour**.

ORIGINAL CERTIFICATION DATE
January 29, 2018

CERTIFICATION RENEWAL RECORD
Renewed annually through December 2023.

METHOD MODIFICATION RECORD
1. **November 2018 Level 1**

SUMMARY OF MODIFICATION
1. **Editorial edits to insert: add logo, correct typographical errors.**

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NONE

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NONE

PRINCIPLE OF THE METHOD (1)

The Wheat/Gluten ELISA kit is a sandwich enzyme linked immunosorbent assay based on anti-gliadin polyclonal antibody. Gliadin is extracted from food samples by heating in boiling water for 10 minutes or shaking overnight with extraction buffer, which includes a detergent and reducing agent. A complex of antibody, antigen, and enzyme-conjugated antibody is formed by a two-step reaction and color development in the presence of enzyme with the addition of substrate. Absorbance is measured at a wavelength of 450 nm after the addition of a stop solution. The concentration of wheat protein/gluten, corresponding to the measured absorbance, can be determined by preparing a wheat protein standard solution curve.

DISCUSSION OF THE VALIDATION STUDY (1)

The Wheat/Gluten ELISA kit showed sufficient performance for wheat/ gluten detection in all of the foods tested. In the cross reactivity study, 37 of the 38 compounds showed negative results and only oat showed a false positive, however, the reactivity of oat was much lower than the threshold of gluten free (20 ppm). It is difficult to get pure oats because of contamination from agricultural distribution. Our oats may have been naturally contaminated with gluten. The protein recovery rate of wheat, rye, and barley can be measured roughly to the same degree suggesting that the Wheat/ Gluten ELISA kit is suitable for gluten monitoring. In the matrix study, recovery was sufficient among the matrixes, regardless of the assay range and extraction method. In addition, the recovery rate in incurred bread was close to the known concentration. The Extraction Buffer contains a surfactant and a reducing agent to increase the efficiency of protein extraction so it should be possible to measure wheat/ gluten in processed foods like bread. Almost all LODs were below the lowest concentration of the standard. The kit was also determined to be robust and stable.

Table 1 Data summary of water samples spiked with gluten (Overnight extraction + Low range assay) (1)

Gluten contamination level, ppm	0	1	2.5	6	10	14
Known concentration, ppm	0.00	0.96	2.40	5.75	9.60	13.44
Mean*, ppm	0.00	0.76	1.94	4.84	8.22	11.70
Mean recovery, %	NA	79	81	84	86	87
Bias, ppm	0.00	-0.20	-0.46	-0.91	-1.38	-1.74
SD	0.00	0.03	0.11	0.24	0.44	0.47
RSDr, %	NA	3.9	5.7	5.0	5.4	4.0

*10 individually extracted samples were tested for each concentration.

Table 2 Data summary of water samples spiked with gluten (Short time extraction + Low range assay) (1)

Gluten contamination level, ppm	0	1	2.5	6	10	14
Known concentration, ppm	0.00	0.96	2.40	5.75	9.60	13.44
Mean*, ppm	0.00	0.77	1.97	4.85	7.94	11.29
Mean recovery, %	NA	80	82	84	83	84
Bias, ppm	0.00	-0.19	-0.43	-0.90	-1.66	-2.15
SD	0.00	0.05	0.12	0.27	0.46	0.74
RSDr, %	NA	6.5	6.1	5.6	5.8	6.6

*10 individually extracted samples were tested for each concentration.

Table 3 Data summary of water samples spiked with gluten (Overnight extraction + High range assay) (1)

Gluten contamination level, ppm	0	10	20	30	40	50
Known concentration, ppm	0.00	9.60	19.19	28.79	38.40	48.00
Mean*, ppm	0.04	8.03	16.05	24.86	33.68	42.04
Mean recovery, %	NA	84	84	86	88	88
Bias, ppm	0.04	-1.57	-3.14	-3.93	-4.72	-5.96
SD	0.09	0.52	0.99	1.60	1.55	2.41
RSDr, %	NA	6.5	6.2	6.4	4.6	5.7

*10 individually extracted samples were tested for each concentration.

Table 4 Data summary of water samples spiked with gluten (Short time extraction + High range assay) (1)

Gluten contamination level, ppm	0	10	20	30	40	50
Known concentration, ppm	0.00	9.60	19.19	28.79	38.40	48.00
Mean*, ppm	0.01	8.32	16.31	24.71	32.69	42.23
Mean recovery, %	NA	87	85	86	85	88
Bias, ppm	0.01	-1.28	-2.88	-4.08	-5.71	-5.77
SD	0.02	0.40	0.78	1.54	1.51	2.31
RSDr, %	NA	4.8	4.8	6.2	4.6	5.5

*10 individually extracted samples were tested for each concentration.

Table 5 Data summary of ice cream samples spiked with gluten (Overnight extraction + Low range assay) (1)

Gluten contamination level, ppm	0	1	2.5	6	10	14
Known concentration, ppm	0.00	0.96	2.40	5.75	9.60	13.44
Mean*, ppm	0.01	0.73	1.91	4.69	7.59	10.93
Mean recovery, %	NA	76	80	82	79	81
Bias, ppm	0.01	-0.23	-0.49	-1.06	-2.01	-2.51
SD	0.01	0.06	0.10	0.28	0.36	0.44
RSDr, %	NA	8.2	5.2	6.0	4.7	4.0

*10 individually extracted samples were tested for each concentration.

Table 6 Data summary of ice cream samples spiked with gluten (Short time extraction + Low range assay) (1)

Gluten contamination level, ppm	0	1	2.5	6	10	14
Known concentration, ppm	0.00	0.96	2.40	5.75	9.60	13.44
Mean*, ppm	0.00	0.73	1.91	4.77	7.80	11.00
Mean recovery, %	NA	76	80	83	81	82
Bias, ppm	0.00	-0.23	-0.49	-0.98	-1.80	-2.44
SD	0.01	0.03	0.11	0.26	0.51	0.68
RSDr, %	NA	4.1	5.8	5.5	6.5	6.2

*10 individually extracted samples were tested for each concentration.

Table 7 Data summary of ice cream samples spiked with gluten (Overnight extraction + High range assay) (1)

Gluten contamination level, ppm	0	10	20	30	40	50
Known concentration, ppm	0.00	9.60	19.19	28.79	38.40	48.00
Mean*, ppm	0.00	8.10	15.96	24.41	32.95	41.43
Mean recovery, %	NA	84	83	85	86	86
Bias, ppm	0.00	-1.50	-3.23	-4.38	-5.45	-6.57
SD	0.01	0.51	1.08	1.86	2.25	2.23
RSDr, %	NA	6.3	6.8	7.6	6.8	5.4

*10 individually extracted samples were tested for each concentration.

Table 8 Data summary of ice cream samples spiked with gluten (Short time extraction + High range assay) (1)

Gluten contamination level, ppm	0	10	20	30	40	50
Known concentration, ppm	0.00	9.60	19.19	28.79	38.40	48.00
Mean*, ppm	0.01	8.12	15.94	24.22	33.13	41.34
Mean recovery, %	NA	85	83	84	86	86
Bias, ppm	0.01	-1.48	-3.25	-4.57	-5.27	-6.66
SD	0.02	0.67	0.74	1.63	1.71	3.19
RSDr, %	NA	8.3	4.6	6.7	5.2	7.7

*10 individually extracted samples were tested for each concentration.

Table 9 Data summary of soup samples spiked with gluten (Overnight extraction + Low range assay) (1)

Gluten contamination level, ppm	0	1	2.5	6	10	14
Known concentration, ppm	0.00	0.96	2.40	5.75	9.60	13.44
Mean*, ppm	0.06	0.85	2.09	5.00	8.36	11.93
Mean recovery, %	NA	89	87	87	87	89
Bias, ppm	0.06	-0.11	-0.31	-0.75	-1.24	-1.51
SD	0.02	0.03	0.12	0.27	0.45	0.70
RSDr, %	NA	3.5	5.7	5.4	5.4	5.9

*10 individually extracted samples were tested for each concentration.

Table 10 Data summary of soup samples spiked with gluten (Short time extraction + Low range assay) (1)

Gluten contamination level, ppm	0	1	2.5	6	10	14
Known concentration, ppm	0.00	0.96	2.40	5.75	9.60	13.44
Mean*, ppm	0.05	0.84	2.06	4.71	7.93	11.35
Mean recovery, %	NA	88	86	82	83	84
Bias, ppm	0.05	-0.12	-0.34	-1.04	-1.67	-2.09
SD	0.02	0.04	0.16	0.29	0.42	0.59
RSDr, %	NA	4.8	7.8	6.2	5.3	5.2

*10 individually extracted samples were tested for each concentration.

Table 11 Data summary of soup samples spiked with gluten (Overnight extraction + High range assay) (1)

Gluten contamination level, ppm	0	10	20	30	40	50
Known concentration, ppm	0.00	9.60	19.19	28.79	38.40	48.00
Mean*, ppm	0.03	8.21	16.94	25.00	33.93	43.28
Mean recovery, %	NA	86	88	87	88	90
Bias, ppm	0.03	-1.39	-2.25	-3.79	-4.47	-4.72
SD	0.04	0.45	1.14	1.70	2.15	3.55
RSDr, %	NA	5.5	6.7	6.8	6.3	8.2

*10 individually extracted samples were tested for each concentration.

Table 12 Data summary of soup samples spiked with gluten (Short time extraction + High range assay) (1)

Gluten contamination level, ppm	0	10	20	30	40	50
Known concentration, ppm	0.00	9.60	19.19	28.79	38.40	48.00
Mean*, ppm	0.03	8.54	16.37	25.59	33.40	42.85
Mean recovery, %	NA	89	85	89	87	89
Bias, ppm	0.03	-1.06	-2.82	-3.20	-5.00	-5.15
SD	0.04	0.63	1.18	1.09	1.53	1.53
RSDr, %	NA	7.4	7.2	4.3	4.6	3.6

*10 individually extracted samples were tested for each concentration.

Table 13 Data summary of water samples spiked with wheat protein (Overnight extraction + Low range assay) (1)

Wheat protein contamination level, ppm	0	1	2.5	6	10	14
Known concentration, ppm	0.00	0.89	2.23	5.34	8.90	12.46
Mean*, ppm	0.01	0.88	2.40	6.11	10.22	13.85
Mean recovery, %	NA	99	108	114	115	111
Bias, ppm	0.01	-0.01	0.17	0.77	1.32	1.39
SD	0.03	0.07	0.12	0.47	0.53	0.88
RSDr, %	NA	8.0	5.0	7.7	5.2	6.4

*10 individually extracted samples were tested for each concentration.

Table 14 Data summary of water samples spiked with wheat protein (Short time extraction + Low range assay) (1)

Wheat protein contamination level, ppm	0	1	2.5	6	10	14
Known concentration, ppm	0.00	0.89	2.23	5.34	8.90	12.46
Mean*, ppm	0.01	0.83	2.47	6.40	10.54	13.98
Mean recovery, %	NA	93	111	120	118	112
Bias, ppm	0.01	-0.06	0.24	1.06	1.64	1.52
SD	0.02	0.07	0.18	0.46	0.82	0.91
RSDr, %	NA	8.4	7.3	7.2	7.8	6.5

*10 individually extracted samples were tested for each concentration.

Table 15 Data summary of water samples spiked with wheat protein (Overnight extraction + High range assay) (1)

Wheat protein contamination level, ppm	0	10	20	30	40	50
Known concentration, ppm	0.00	8.90	17.81	26.70	35.60	44.51
Mean*, ppm	0.00	10.03	21.84	32.63	42.96	52.37
Mean recovery, %	NA	113	123	122	121	118
Bias, ppm	0.00	1.13	4.03	5.93	7.36	7.86
SD	0.01	0.78	0.96	1.52	1.57	1.27
RSDr, %	NA	7.8	4.4	4.7	3.7	2.4

*10 individually extracted samples were tested for each concentration.

Table 16 Data summary of water samples spiked with wheat protein (Short time extraction + High range assay) (1)

Wheat protein contamination level, ppm	0	10	20	30	40	50
Known concentration, ppm	0.00	8.90	17.81	26.70	35.60	44.51
Mean*, ppm	0.02	10.46	21.58	33.32	44.85	53.34
Mean recovery, %	NA	118	121	125	126	120
Bias, ppm	0.02	1.56	3.77	6.62	9.25	8.83
SD	0.04	0.46	1.32	1.78	2.09	4.05
RSDr, %	NA	4.4	6.1	5.3	4.7	7.6

*10 individually extracted samples were tested for each concentration.

Table 17 Data summary of cider samples spiked with wheat protein (Overnight extraction + Low range assay) (1)

Wheat protein contamination level, ppm	0	1	2.5	6	10	14
Known concentration, ppm	0.00	0.89	2.23	5.34	8.90	12.46
Mean*, ppm	0.00	0.83	2.32	5.80	10.76	13.96
Mean recovery, %	NA	93	104	109	121	112
Bias, ppm	0.00	-0.06	0.09	0.46	1.86	1.50
SD	0.00	0.10	0.15	0.50	0.49	0.70
RSDr, %	NA	12.0	6.5	8.6	4.6	5.0

*10 individually extracted samples were tested for each concentration.

Table 18 Data summary of cider samples spiked with wheat protein (Short time extraction + Low range assay) (1)

Wheat protein contamination level, ppm	0	1	2.5	6	10	14
Known concentration, ppm	0.00	0.89	2.23	5.34	8.90	12.46
Mean*, ppm	0.00	0.83	2.39	5.59	10.48	14.05
Mean recovery, %	NA	93	107	105	118	113
Bias, ppm	0.00	-0.06	0.16	0.25	1.58	1.59
SD	0.01	0.05	0.18	0.46	0.61	1.15
RSDr, %	NA	6.0	7.5	8.2	5.8	8.2

*10 individually extracted samples were tested for each concentration.

Table 19 Data summary of cider samples spiked with wheat protein (Overnight extraction + High range assay) (1)

Wheat protein contamination level, ppm	0	10	20	30	40	50
Known concentration, ppm	0.00	8.90	17.81	26.70	35.60	44.51
Mean*, ppm	0.02	10.61	21.60	33.05	42.69	54.19
Mean recovery, %	NA	119	121	124	120	122
Bias, ppm	0.02	1.71	3.79	6.35	7.09	9.68
SD	0.05	0.54	1.18	1.99	2.80	2.76
RSDr, %	NA	5.1	5.5	6.0	6.6	5.1

*10 individually extracted samples were tested for each concentration.

Table 20 Data summary of cider samples spiked with wheat protein (Short time extraction + High range assay) (1)

Wheat protein contamination level, ppm	0	10	20	30	40	50
Known concentration, ppm	0.00	8.90	17.81	26.70	35.60	44.51
Mean*, ppm	0.02	10.21	21.18	32.36	44.65	54.45
Mean recovery, %	NA	115	119	121	125	122
Bias, ppm	0.02	1.31	3.37	5.66	9.05	9.94
SD	0.03	0.42	1.35	1.99	2.73	3.33
RSDr, %	NA	4.1	6.4	6.1	6.1	6.1

*10 individually extracted samples were tested for each concentration.

Table 21 Data summary of rice flour samples spiked with wheat protein (Overnight extraction + Low range assay) (1)

Wheat protein contamination level, ppm	0	1	2.5	6	10	14
Known concentration, ppm	0.00	0.89	2.23	5.34	8.90	12.46
Mean*, ppm	0.12	0.99	2.70	6.57	11.46	14.80
Mean recovery, %	NA	111	121	123	129	119
Bias, ppm	0.12	0.10	0.47	1.23	2.56	2.34
SD	0.04	0.10	0.23	0.21	0.55	0.64
RSDr, %	NA	10.1	8.5	3.2	4.8	4.3

*10 individually extracted samples were tested for each concentration.

Table 22 Data summary of rice flour samples spiked with wheat protein (Short time extraction + Low range assay) (1)

Wheat protein contamination level, ppm	0	1	2.5	6	10	14
Known concentration, ppm	0.00	0.89	2.23	5.34	8.90	12.46
Mean*, ppm	0.02	0.99	2.87	7.21	12.59	16.73
Mean recovery, %	NA	111	129	135	141	134
Bias, ppm	0.02	0.10	0.64	1.87	3.69	4.27
SD	0.02	0.10	0.28	0.49	0.86	0.98
RSDr, %	NA	10.1	9.8	6.8	6.8	5.9

*10 individually extracted samples were tested for each concentration.

Table 23 Data summary of rice flour samples spiked with wheat protein (Overnight extraction + High range assay) (1)

Wheat protein contamination level, ppm	0	10	20	30	40	50
Known concentration, ppm	0.00	8.90	17.81	26.70	35.60	44.51
Mean*, ppm	0.03	11.09	24.28	34.50	47.00	57.59
Mean recovery, %	NA	125	136	129	132	129
Bias, ppm	0.03	2.19	6.47	7.80	11.40	13.08
SD	0.04	0.73	1.24	2.29	3.81	2.62
RSDr, %	NA	6.6	5.1	6.6	8.1	4.5

*10 individually extracted samples were tested for each concentration.

Table 24 Data summary of rice flour samples spiked with wheat protein (Short time extraction + High range assay) (1)

Wheat protein contamination level, ppm	0	10	20	30	40	50
Known concentration, ppm	0.00	8.90	17.81	26.70	35.60	44.51
Mean*, ppm	0.01	12.66	24.40	38.16	51.16	62.41
Mean recovery, %	NA	142	137	143	144	140
Bias, ppm	0.01	3.76	6.59	11.46	15.56	17.90
SD	0.02	0.92	1.99	2.15	3.98	3.09
RSDr, %	NA	7.3	8.2	5.6	7.8	5.0

*10 individually extracted samples were tested for each concentration.

Table 25 LOD and LOQ determination of water samples spiked with gluten (1)

Extraction method	Overnight	Short time	Overnight	Short time
Assay range	Low	Low	High	High
	0.00	0.00	0.00	0.00
	0.00	0.00	0.00	0.04
	0.08	0.00	0.00	0.00
	0.00	0.05	0.00	0.12
	0.00	0.00	0.00	0.01
	0.00	0.00	0.00	0.00
	0.01	0.00	0.00	0.00
	0.01	0.00	0.00	0.00
	0.00	0.00	0.00	0.00
	0.00	0.00	0.03	0.00
Mean(n=10), ppm	0.01	0.01	0.00	0.02
Sr	0.025	0.016	0.009	0.038
LOD, ppm	0.08	0.05	0.03	0.13
LOQ, ppm	0.25	0.16	0.09	0.38

Table 26 LOD and LOQ determination of ice cream samples spiked with gluten (1)

Extraction method	Overnight	Short time	Overnight	Short time
Assay range	Low	Low	High	High
	0.01	0.00	0.00	0.00
	0.00	0.00	0.00	0.00
	0.00	0.00	0.03	0.00
	0.02	0.00	0.00	0.00
	0.00	0.00	0.00	0.03
	0.00	0.00	0.00	0.00
	0.00	0.00	0.00	0.00
	0.00	0.00	0.00	0.00
	0.00	0.00	0.00	0.00
	0.04	0.03	0.00	0.04
Mean(n=10), ppm	0.01	0.00	0.00	0.01
Sr	0.013	0.009	0.009	0.015
LOD, ppm	0.04	0.03	0.03	0.05
LOQ, ppm	0.13	0.09	0.09	0.15

Table 27 LOD and LOQ determination of soup samples spiked with gluten (1)

Extraction method	Overnight	Short time	Overnight	Short time
Assay range	Low	Low	High	High
	0.05	0.04	0.00	0.00
	0.06	0.07	0.07	0.06
	0.10	0.05	0.00	0.08
	0.08	0.06	0.07	0.08
	0.04	0.05	0.00	0.00
	0.05	0.05	0.00	0.00
	0.05	0.03	0.00	0.00
	0.05	0.06	0.00	0.00
	0.03	0.04	0.00	0.00
	0.08	0.08	0.12	0.11
Mean(n=10), ppm	0.06	0.05	0.03	0.03
Sr	0.021	0.015	0.044	0.044
LOD, ppm	0.07	0.05	0.15	0.15
LOQ, ppm	0.21	0.15	0.44	0.44

Table 40 LOD and LOQ determination of cider samples spiked with wheat protein (1)

Extraction method	Overnight	Short time	Overnight	Short time
Assay range	Low	Low	High	High
	0.00	0.00	0.00	0.00
	0.02	0.00	0.00	0.00
	0.00	0.00	0.10	0.04
	0.00	0.00	0.13	0.07
	0.02	0.00	0.00	0.07
	0.00	0.00	0.00	0.00
	0.00	0.00	0.00	0.00
	0.00	0.00	0.00	0.00
	0.00	0.00	0.00	0.00
	0.00	0.02	0.00	0.00
Mean(n=10), ppm	0.00	0.00	0.02	0.02
Sr	0.008	0.006	0.049	0.030
LOD, ppm	0.03	0.02	0.16	0.10
LOQ, ppm	0.08	0.06	0.49	0.30

Table 41 LOD and LOQ determination of rice flour samples spiked with wheat protein (1)

Extraction method	Overnight	Short time	Overnight	Short time
Assay range	Low	Low	High	High
	0.00	0.00	0.00	0.00
	0.04	0.07	0.05	0.00
	0.04	0.00	0.04	0.02
	0.01	0.02	0.04	0.02
	0.01	0.00	0.13	0.07
	0.01	0.00	0.00	0.00
	0.00	0.00	0.00	0.00
	0.01	0.03	0.00	0.00
	0.01	0.03	0.00	0.00
	0.01	0.01	0.03	0.00
Mean(n=10), ppm	0.01	0.02	0.03	0.01
Sr	0.014	0.023	0.041	0.022
LOD, ppm	0.05	0.07	0.14	0.07
LOQ, ppm	0.14	0.22	0.41	0.22

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