U.S. DEPARTMENT OF COMMERCE
NATIONAL INSTITUTE OF STANDARDS AND TECHNOLOGY
ORGANIC ANALYTICAL RESEARCH DIVISION
GAITHERSBURG, MARYLAND 20899

SIM.8.P5.2 Study Report: Intercomparison of Milk Powder and Infant Formula Analyses

July 3, 2002

As part of Inter-American Metrology System (SIM) intercomparison SIM.QM-P5 in 1999/2000, 15 laboratories measured vitamins A and E, linoleic acid, linolenic acid, calcium, sodium, iron, and zinc in Standard Reference Material (SRM) 1846 Infant Formula. If a laboratory had access to the Certificate of Analysis for this material, it was possible to learn what the "right" answers should be, and a follow-up exercise (SIM.8.P5.2) was organized, involving a milk powder as the test sample and SRM 1846 as a control material. SIM Country Coordinators were asked to identify up to three interested participants in their countries. The Action Plan to which these laboratories responded is provided in Appendix A.

Samples were shipped to ten country coordinators (Appendix B), who were asked (Appendix C) to distribute the samples and accompanying materials to the participating laboratories within their countries. Twenty-two laboratories reported results. Participants were provided with a description of the analytical protocol, data sheets for reporting results, and sheets for reporting their analytical methods and the methods they used for calculating expanded uncertainties (Appendix D). Participants were asked to analyze duplicate samples from each of three packets of milk powder for vitamin A, vitamin C, linoleic acid, linolenic acid, calcium, and iron. Participants were asked to analyze single samples from each of three packets of SRM 1846 Infant Formula, and were provided with the material's Certificate of Analysis. Participants were advised not to use SRM 1846 as a calibrant, but rather to use it to evaluate the accuracy of their results for the test sample. (In the case of vitamin A, participants were warned in the cover letter and on the data sheets that, depending on their methods of sample preparation, SRM 1846 may not be a useful control material for vitamin A analysis.[1])

Results for the six analytes are provided in Tables 1 through 6. Consensus values for each of the analytes were not calculated. As noted previously, participants were asked to report their individual values, means, and expanded uncertainties; significant figures are tabulated as they were reported by the participants. Means and *standard deviations* were

[1] Since 1996, when SRM 1846 became available, some laboratories have reported difficulty in extracting vitamin A from it. Over time, it has become increasingly difficult to extract the retinyl palmitate from the microbeadlets in which it is incorporated – hence the warning that SRM 1846 may not be a useful control material for vitamin A in this exercise. Incomplete extraction probably accounts for the low values reported by some of the participants in this intercomparison. Some participants *were* able to obtain the reference value for vitamin A in SRM 1846, implying that the concentration has remained stable.

calculated by the study coordinator, and are also tabulated. These standard deviations were compared to the uncertainties reported by the participants; values are italicized in cases where the two were the same. (For this type of analysis, the standard uncertainty of the sample measurements is frequently representative of the combined standard uncertainty (not the *expanded uncertainty*), as most of the variability in the measurement arises from measurement of the samples themselves rather than calibration or other factors. A SIM workshop on calculation of uncertainties was held in Chile in October 2001, but participants in this intercomparison would not likely have attended.) Certified and reference values in the SRM for the analytes of interest are also provided in Tables 1 through 6. The methods sheets returned by the participants, which include an explanation of their uncertainty calculations, are provided in Appendix E. (Laboratories are identified by code numbers; identities of these code numbers are provided in Appendix F, which is not intended for public release.)

Plots of the participants' results for both the milk powder and the SRM 1846 and the assigned values for SRM 1846 are provided in Figures 1 through 6. The error bars on the assigned values represent expanded uncertainties as reported by the participants, except in the cases labeled "sd," where one standard deviation (either reported by the participant or calculated by the study coordinator, as noted in the figure caption) has been plotted. Dashed lines are drawn across the plots of SRM 1846 data to indicate the boundaries of the expanded uncertainties on the assigned values.

Conclusions and Recommendations:

Many of the participating laboratories performed well in this intercomparison, however there are some notable exceptions. Results can be used by the participants to evaluate whether their methods of analysis are adequate to address the needs of their countries. For example, in the United States, nutrition labeling laws require that processed foods be accurately labeled, but allow for a 20% difference between the true value and label information in the direction of no nutritional harm. (That is, the true value for calcium can be 20% higher than the value provided on the package label, and the consumer is not being misled into believing that there is more calcium present in the food than there actually is.) While a 20% difference in results may be acceptable for an analytical testing laboratory, it may not be an appropriate level of accuracy for a national metrology institute. Individual countries must determine what is an appropriate level of performance to address their own needs.

No immediate follow-up study is planned. Should the need arise, a new study can be organized.

Prepared by:

Katherine E. Sharpless, Ph.D.
Research Chemist

Reviewed by:

Lane C. Sander, Ph.D.
Research Chemist

Stephen A. Wise, Ph.D.
Supervisory Research Chemist

Table 1. Results for vitamin A (μg/g) in milk powder and SRM 1846. Participants were asked to report their individual results, means, and expanded uncertainties (exp uncert), which are shown below. The study coordinator's (SC's) calculated mean and standard deviation (sd) are also shown. Values are italicized if the uncertainty reported by the participant agrees with the standard deviation calculated by the study coordinator. If a participant failed to report an uncertainty (nr), the standard deviation calculated by the study coordinator has been plotted in the figures in this report.

	MILK POWDER			SRM 1846		
Lab	Vitamin A	Reported mean & exp uncert	SC's calculated mean & sd	Vitamin A	Reported mean & exp uncert	SC's calculated mean & sd
3	3.80	3.88	3.88	3.43	4.52	4.52
	3.80	0.08	0.07	4.40	2.88	1.16
	3.86			5.74		
	3.91					
	3.99					
	3.89					
4	2.49	2.24	2.24	2.14	2.51	2.52
	2.24	0.36	0.16	2.66	0.68	0.33
	2.20			2.75		
	2.34					
	2.04					
	2.11					
6	4.07	4.20	4.20	5.23	5.16	5.16
	4.12	0.71	0.10	5.03	0.88	0.12
	4.25			5.23		
	4.14					
	4.28					
	4.31					
10	6.624	5.61	5.83	6.753	7.14	7.14
	5.228	1.97	1.14	7.036	1.97	0.44
	7.544			7.616		
	6.056					
	5.164					
	4.383					
13	27.76	31.85	31.86	40.29	36.73	36.73
	29.10	*5.53*	*5.53*	32.39	*4.01*	*4.01*
	38.50			37.52		
	39.39					
	28.73					
	27.65					

	MILK POWDER			SRM 1846		
Lab	Vitamin A	Reported mean & exp uncert	SC's calculated mean & sd	Vitamin A	Reported mean & exp uncert	SC's calculated mean & sd
18	7.62	7.56	7.56	6.22	7.28	7.28
	8.54	0.66	0.71	7.64	1.12	0.93
	7.78			7.97		
	7.75					
	7.3					
	6.36					
19	2.317	2.564	2.56	3.213	3.57	3.57
	2.401	nr	0.31	3.251	nr	0.59
	3.032			4.256		
	2.839					
	2.261					
	2.532					
NIST	4.48	4.24	4.24	3.68	3.50	3.50
	4.09	0.28	0.24	3.39	0.39	0.16
	3.89			3.43		
	3.99					
	4.51					
	4.44					
	4.23					
	4.41					
	4.44					
	4.4					
	3.86					
	4.13					
23	4.23	4.50	4.50	4.94	5.14	5.14
	4.51	1.26	0.30	5.08	1.24	0.24
	4.92			5.40		
	4.33					
24	4.36	4.23	4.23	5.53	5.52	5.52
	4.12	0.86	0.08	5.73	0.93	0.22
	4.27			5.29		
	4.22					
	4.20					
	4.19					

	MILK POWDER				SRM 1846		
Lab	Vitamin A	Reported mean & exp uncert	SC's calculated mean & sd		Vitamin A	Reported mean & exp uncert	SC's calculated mean & sd
25	4.43	4.46	4.46		5.55	5.47	5.47
	4.48	0.80	0.04		5.40		0.08
	4.48				5.45		
	4.49						
	4.40						
	4.50						
					Reference value:	5.84 " 0.68	

Table 2. Results for vitamin C (μg/g) in milk powder and SRM 1846. Participants were asked to report their individual results, means, and expanded uncertainties (exp uncert), which are shown below. The study coordinator's (SC's) calculated mean and standard deviation (sd) are also shown. Values are italicized if the uncertainty reported by the participant agrees with the standard deviation calculated by the study coordinator. If a participant failed to report a mean and an uncertainty (nr), the mean and standard deviation calculated by the study coordinator have been plotted in the figures in this report.

	MILK POWDER			SRM 1846		
Lab	Vitamin C	Reported mean & exp uncert	SC's calculated mean & sd	Vitamin C	Reported mean & exp uncert	SC's calculated mean & sd
3	204	216	216	1077	1036	1036
	235	18	17	985	116	47
	204			1045		
	235					
	195					
	222					
4	206	242	242	826	859	859
	234	*51*	*51*	897	42	36
	200			854		
	237					
	236					
	340					
6	223	228	228	1107	1105	1105
	226	30	6	1112	144	8
	227			1096		
	227					
	240					
	224					
7	266.63	nr	218.55	1047.79	1047.2	1047.20
	268.6	nr	38.10	1047.61	*0.8706*	*0.87*
	191.12			1046.2		
	191.6					
	195.11					
	198.24					

	MILK POWDER			SRM 1846		
Lab	Vitamin C	Reported mean & exp uncert	SC's calculated mean & sd	Vitamin C	Reported mean & exp uncert	SC's calculated mean & sd
13	0.21	0.18	0.18	1.00	0.99	0.99
	0.21	*0.02*	*0.02*	0.99	*0.01*	*0.01*
	0.17			0.99		
	0.17					
	0.17					
	0.17					
14	839.23	nr	837.82	1235.44	1194.63	1194.63
	864.05	nr	36.43	1189.89	101.82	38.65
	795.58			1158.57		
	815.90					
	894.91					
	817.25					
16	213.13	nr	270.02	1121.25	1133.23	1133.23
	214.31	nr	93.59	1161.37	0.224% RSD	24.46
	389.64			1117.07	(2.54)	
	391.82					
	203.22					
	208.01					
19	357.161	411.18	411.18	1151.266	1153.89	1153.89
	363.976	nr	65.13	1162.500	nr	7.65
	494.53			1147.900		
	494.63					
	378.20					
	378.576					
NIST	296	291	290.75	1195	1149	1149
	292	22	18.48	1079	151	48
	340			1132		
	302			1211		
	300			1145		
	288			1132		
	283					
	284					
	267					
	279					
	279					
	279					

	MILK POWDER				SRM 1846		
Lab	Vitamin C	Reported mean & exp uncert	SC's calculated mean & sd		Vitamin C	Reported mean & exp uncert	SC's calculated mean & sd
23	177	nr	228.25		1088	1079	1079
	164	nr	69.26		1067	112	11
	308				1081		
	264						
25	231	234.4	235		1188	1200	1200
	232	91	3		1201	nr	12
	236				1211		
	240						
	234						
	234						
					Certified value:	1146 " 66	

Table 3. Results for calcium (μg/g) in milk powder and SRM 1846. Participants were asked to report their individual results, means, and expanded uncertainties (exp uncert), which are shown below. The study coordinator's (SC's) calculated mean and standard deviation (sd) are also shown. Values are italicized if the uncertainty reported by the participant agrees with the standard deviation calculated by the study coordinator. If a participant failed to report a mean and an uncertainty (nr), the mean and standard deviation calculated by the study coordinator have been plotted in the figures in this report.

	MILK POWDER			SRM 1846		
Lab	Calcium	Reported mean & exp uncert	SC's calculated mean & sd	Calcium	Reported mean & exp uncert	SC's calculated mean & sd
1	7282	7353	7353	3405	3603	3603
	7480	529	87	3889	260	254
	7336			3515		
	7315					
2	6961	6908	6908	3597	3612	3612
	6955	95	44	3619		13
	6895			3621		
	6847					
	6880					
	6910					
3	5160	5927	5927	3765	3691	3691
	5480	1069	494	3663	204	64
	6029			3646		
	6217					
	6317					
	6358					
4	6838	6828	6828	3641	3625	3625
	6843	45	15	3631	*20*	*20*
	6831			3603		
	6800					
	6832					
	6826					
5	7150	7120	7115	3660	3620	3617
	7180	270	73	3560	140	51
	7100			3630		
	7000					
	7190					
	7070					

	MILK POWDER				SRM 1846		
Lab	Calcium	Reported mean & exp uncert	SC's calculated mean & sd		Calcium	Reported mean & exp uncert	SC's calculated mean & sd
7	2294.46	nr	1953.76		1520.34	1519.9	1519.91
	2291.42	nr	383.28		1519.2	*0.6171*	*0.62*
	1472.84				1520.18		
	1471.62						
	2104.41						
	2087.82						
8	6990	6992	6992		3541	3572	3572
	7009	100	31		3580	40	28
	7004				3596		
	6931						
	7012						
	7007						
9	7685	7592	7592		3849	3822	3822
	7593	nr	51		3813		24
	7560				3804		
	7602						
	7536						
	7574						
10	7060	7023	7023		3494	3518	3516
	6958	583	46		3493	303	39
	7070				3561		
	7049						
	6977						
	7024						
11	6844	6735	6735		3570	3500	3500
	6794	*132.4*	*132.4*		3402	87.4	87
	6824				3528		
	6697						
	6766						
	6486						
13	6500	6705	6705		3470	3425	3425
	6600	*140*	*140*		3380	45	45
	6780				3425		
	6690						
	6890						
	6770						

	MILK				SRM		

	POWDER				1846		
Lab	Calcium	Reported mean & exp uncert	SC's calculated mean & sd		Calcium	Reported mean & exp uncert	SC's calculated mean & sd
14	7320.50	nr	7496.53		3619.51	3647.42	3647.42
	7512.40	nr	198.77		3631.98	100.05	38.06
	7422.40				3690.78		
	7281.20						
	7803.10						
	7639.60						
16	7599.45	nr	6652.24		3797.47	3858.91	3858.91
	7767.18	nr	812.77		3802.83	21.93% RSD	101.80
	5936.62				3976.42	(846.26)	
	6056.13						
	6194.38						
	6359.68						
17	6809	nr	5858		3299	nr	3294
	6523	nr	633		3052	nr	241
	5427				3533		
	5428						
	5459						
	5500						
18a	6902.16	6928.49	6928.49		3695.96	3693.38	3693.38
	6869.64	214.34	47.90		3676.14	121.82	17.05
	6982.44				3683.83		
	6915.61				3690.08		
	6972.58				3720.88		
18b	7207.47	7107.19	7107.19		3630.91	3625.51	3625.51
	7171.40	249.23	81.81		3576.31	129.98	39.98
	7009.06				3619.16		
	7093.53				3686.95		
	7054.48				3614.21		
19	7227.02	7257.05	7257.05		3460.06	3454.16	3454.16
	7270.92	nr	32.99		3446.43	nr	7.00
	7247.89				3455.99		
	7213.56						
	7292.90						
	7290.02						

	MILK POWDER				SRM 1846		
Lab	Calcium	Reported mean & exp uncert	SC's calculated mean & sd		Calcium	Reported mean & exp uncert	SC's calculated mean & sd
NIST	7331	7302	7302		3731	3711	3711
	7280	51	24		3702	37	18
	7284				3699		
	7306						
	7329						
	7282						
25	8014	7998	7998		3860	3863	3863
	7984	198	16		3867	nr	4
	7988				3863		
	7981						
	8002						
	8018						
					Reference value:	3670 " 200	

Table 4. Results for iron (μg/g) in milk powder and SRM 1846. Participants were asked to report their individual results, means, and expanded uncertainties (exp uncert), which are shown below. The study coordinator's (SC's) calculated mean and standard deviation (sd) are also shown. Values are italicized if the uncertainty reported by the participant agrees with the standard deviation calculated by the study coordinator. If a participant failed to report a mean and an uncertainty (nr), the mean and standard deviation calculated by the study coordinator have been plotted in the figures in this report.

	MILK POWDER				SRM 1846		
Lab	Iron	Reported mean & exp uncert	SC's calculated mean & sd		Iron	Reported mean & exp uncert	SC's calculated mean & sd
1	100.2	106.6	106.6		67.7	67.1	67.1
	110.2	7.7	4.4		68.1	4.8	1.3
	107.7				65.6		
	108.1						
2	102.8	102.9	102.9		59.7	59.4	59.4
	103.5	2.0	0.6		59.4	nr	0.4
	103.2				59.0		
	101.9						
	102.7						
	103.1						
3	96.9	90.9	90.9		63.4	64.1	64.1
	93.9	9.4	4.3		64.5	2.1	0.6
	92.5				64.4		
	89.7						
	87.5						
	85.1						
4	101	102	102		61	61	61
	103	*1*	*1*		63	1	2
	102				60		
	101						
	102						
	102						
5	107	105	105		63.5	63.0	63.0
	107	2.9	1.5		62.2	2.1	0.7
	104				63.3		
	105						
	104						
	104						

	MILK POWDER				SRM 1846		
Lab	Iron	Reported mean & exp uncert	SC's calculated mean & sd		Iron	Reported mean & exp uncert	SC's calculated mean & sd
7	127.14	nr	89.06		66.05	66.06	66.06
	128.86	nr	40.66		66.1	3.6055	0.04
	40.43				66.03		
	37.82						
	99.06						
	101.03						
8	117	112	112		64	66	66
	111	3	3		68	3	2
	113				67		
	112						
	112						
	108						
9	113	114	114		64	70	70
	119	nr	6		72	nr	5
	119				74		
	118						
	107						
	108						
10	107.3	108.8	108.8		67.2	65.0	65.0
	108.9	4	2.0		63.3	4.0	2.0
	109.7				64.4		
	109.7						
	105.8						
	111.3						
11	121.3	117.1	117.1		81.9	81.5	81.5
	112.1	*3.64*	*3.64*		81.0	*0.5*	*0.5*
	116.4				81.7		
	114.1						
	120.7						
	118.1						
12	101.4	101.5	101.5		64.4	64.6	64.6
	101.6	3.?	0.8		64.9	2.?	0.2
	101.3	(illegible			64.6	(illegible	
	102.8	on fax; no)				on fax; no)	
	101.6	response to				response to	
	100.3	e-mail query)				e-mail query)	

	MILK						

	POWDER				SRM 1846		
Lab	Iron	Reported mean & exp uncert	SC's calculated mean & sd		Iron	Reported mean & exp uncert	SC's calculated mean & sd
13	101.4	96.0	96.0		63.0	64.0	64.0
	93.0	*2.9*	*2.9*		63.8	*1.1*	*1.1*
	94.3				65.1		
	95.4						
	95.5						
	96.5						
14	122.20	nr	131.43		59.27	59.55	59.55
	120.00	nr	8.67		59.34	1.53	0.43
	140.20				60.05		
	140.30						
	133.10						
	132.80						
16	120.29	nr	119.29		56.16	56.62	56.62
	122.49	nr	4.89		56.66	14.95% RSD	0.44
	113.36				57.04	(2.803)	
	112.98						
	122.62						
	124.02						
17	103	nr	102		67.6	nr	68.0
	107	nr	6		69.2	nr	1.1
	96				67.2		
	109						
	94						
	105						
18a	106.07	105.07	105.07		64.05	62.46	62.45
	106.24	3.65	1.41		62.21	2.17	1.01
	102.89				62.33		
	105.70				62.44		
	104.44				61.24		
18b	103.70	104.79	104.79		63.03	62.56	62.56
	104.77	5.11	0.76		61.89	2.84	1.22
	105.06				64.50		
	104.63				61.66		
	105.80				61.74		

	MILK POWDER				SRM 1846		
Lab	Iron	Reported mean & exp uncert	SC's calculated mean & sd		Iron	Reported mean & exp uncert	SC's calculated mean & sd
19	100.026	100.386	100.386		61.390	61.31	61.31
	100.029	nr	0.461		61.089		0.19
	100.761				61.449		
	100.103						
	100.256						
	101.141						
NIST	110.3	105.4	105.4		62.0	61.8	61.8
	104.0	2.6	2.5		62.4	1.4	0.7
	103.7				61.0		
	105.4						
	105.2						
	104.0						
					Reference value:	63.1 " 4.0	

Table 5. Results for linoleic acid (%) in milk powder and SRM 1846. Participants were asked to report their individual results, means, and expanded uncertainties (exp uncert), which are shown below. The study coordinator's (SC's) calculated mean and standard deviation (sd) are also shown. Values are italicized if the uncertainty reported by the participant agrees with the standard deviation calculated by the study coordinator. If a participant failed to report a mean and an uncertainty (nr), the mean and standard deviation calculated by the study coordinator have been plotted in the figures in this report. Fatty acids were reported as free fatty acids, fatty acid methyl esters (FAMEs), and triglycerides. Means and uncertainties, as reported by the participants when possible, were converted to triglycerides if they were originally reported as free fatty acids or FAMEs. If means and/or uncertainties were not reported by the participant, the conversion to triglycerides was performed on the means and standard deviations calculated by the study coordinator.

		MILK POWDER				INFANT FORMULA			
Lab	Reported as:	Linoleic Acid	Reported mean & exp uncert	SC's calculated mean & sd	As the triglyceride	Linoleic Acid	Reported mean & exp uncert	SC's calculated mean & sd	As the triglyceride
3	Triglycerides	4.87	4.98	4.91	4.98	3.49	3.38	3.39	3.38
		4.89	0.070	0.068	0.070	3.38	0.2	0.10	0.2
		4.92				3.30			
		5.00							
		4.82							
		4.98							
4	FAMEs	4.77	4.91	4.91	4.88	3.57	3.63	3.63	3.61
		4.80	0.25	0.12	0.25	3.64	0.11	0.05	0.11
		4.96				3.67			
		5.09							
		4.98							
		4.85							

		MILK POWDER				INFANT FORMULA			
Lab	Reported as:	Linoleic Acid	Reported mean & exp uncert	SC's calculated mean & sd	As the triglyceride	Linoleic Acid	Reported mean & exp uncert	SC's calculated mean & sd	As the triglyceride
13	FAMEs	24.11	24.11	24.11	24.00	3.81	3.57	3.57	3.55
		24.31	*0.13*	*0.13*	0.13	3.43	*0.21*	*0.21*	0.21
		24.19				3.46			
		24.00							
		23.96							
		24.09							
18	FAMEs	4.322	4.296	4.30	4.2764	3.349	3.339	3.34	3.324
		4.399	0.074	0.090	0.074	3.358	0.0295	0.026	0.029
		4.293				3.310			
		4.131							
		4.342							
		4.290							
19	Free fatty acids	4.44	4.63	4.63	4.84	3.49	3.50	3.51	3.66
		4.54	nr	0.197	nr	3.49	nr	0.03	
		4.66				3.54			
		4.41							
		4.85							
		4.86							

		MILK POWDER				INFANT FORMULA			
Lab	Reported as:	Linoleic Acid	Reported mean & exp uncert	SC's calculated mean & sd	As the triglyceride	Linoleic Acid	Reported mean & exp uncert	SC's calculated mean & sd	As the triglyceride
NIST	Triglycerides	5.008	5.205	5.20	5.205	4.071	4.079	4.079	4.079
		5.161	0.210	0.182	0.210	4.062	0.171	0.060	0.171
		5.561				4.164			
		5.147				4.029			
		5.073				4.010			
		5.372				4.136			
		5.017							
		5.157							
		5.489							
		5.070							
		5.132							
		5.272							
23	Triglycerides	4.50	4.54	4.54	4.54	3.38	3.48	3.48	3.48
		4.56	0.56	0.14	0.56	3.60	0.42	0.11	0.42
		4.66				3.46			
		4.74							
		4.42							
		4.38							

		MILK POWDER					INFANT FORMULA			
Lab	Reported as:	Linoleic Acid	Reported mean & exp uncert	SC's calculated mean & sd	As the triglyceride		Linoleic Acid	Reported mean & exp uncert	SC's calculated mean & sd	As the triglyceride
24	Triglycerides	4.71	4.70	4.70	4.70		3.74	3.73	3.73	3.73
		4.72	0.63	0.02	0.63		3.70	0.50	0.02	0.50
		4.71					3.74			
		4.71								
		4.68								
		4.68								
							Reference value:	3.48 "± 0.40		

Table 6. Results for linolenic acid (%) in milk powder and SRM 1846. Participants were asked to report their individual results, means, and expanded uncertainties (exp uncert), which are shown below. The study coordinator's (SC's) calculated mean and standard deviation (sd) are also shown. Values are italicized if the uncertainty reported by the participant agrees with the standard deviation calculated by the study coordinator. If a participant failed to report a mean and an uncertainty (nr), the mean and standard deviation calculated by the study coordinator have been plotted in the figures in this report. Fatty acids were reported as free fatty acids, fatty acid methyl esters (FAMEs), and triglycerides. Means and uncertainties, as reported by the participants when possible, were converted to triglycerides if they were originally reported as free fatty acids or FAMEs. If means and/or uncertainties were not reported by the participant, the conversion to triglycerides was performed on the means and standard deviations calculated by the study coordinator.

		MILK POWDER					INFANT FORMULA			
Lab	Reported as:	Linolenic Acid	Reported mean & exp uncert	SC's calculated mean & sd	As the triglyceride		Linolenic Acid	Reported mean & exp uncert	SC's calculated mean & sd	As the triglyceride
3	Triglycerides	0.16	0.19	0.18	0.19		0.089	0.088	0.090	0.088
		0.19	0.02	0.01	0.02		0.089	0.009	0.001	0.009
		0.17					0.091			
		0.19								
		0.18								
		0.19								
4	FAMEs	0.19	0.19	0.19	0.19		0.08	0.08	0.08	0.08
		0.19	0.02	0.01	0.02		0.08	0.0009	0.0000	0.0009
		0.19					0.08			
		0.21								
		0.19								
		0.19								

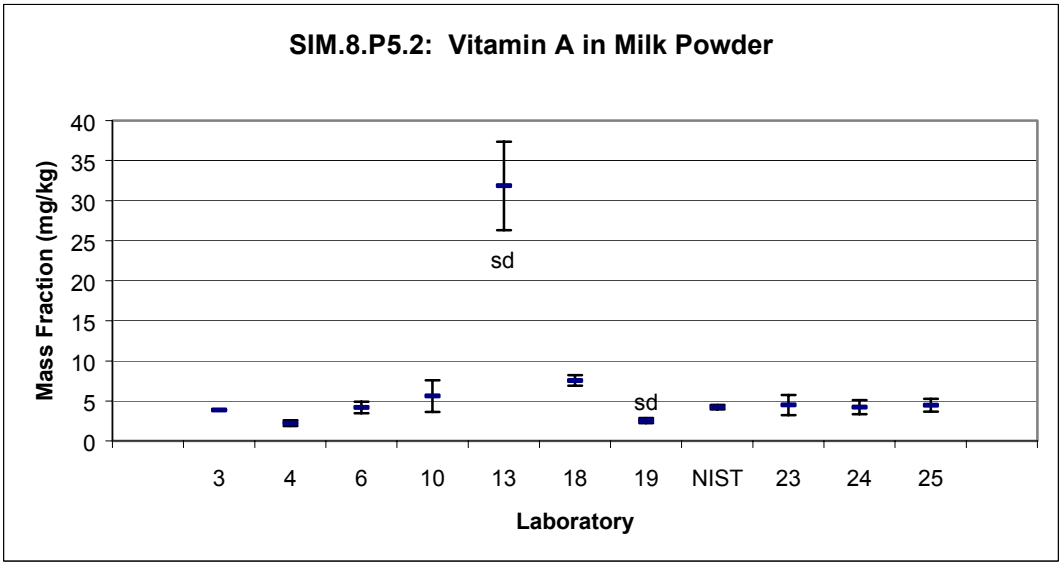
		MILK POWDER					INFANT FORMULA			
Lab	Reported as:	Linolenic Acid	Reported mean & exp uncert	SC's calculated mean & sd	As the triglyceride		Linolenic Acid	Reported mean & exp uncert	SC's calculated mean & sd	As the triglyceride
13	FAMEs	0.74	0.74	0.74	0.74		0.0837	0.0828	0.0828	0.0824
		0.75	0.0119	0.014	0.01		0.0837	*0.0016*	*0.0016*	0.0016
		0.72					0.0810			
		0.72								
		0.74								
		0.75								
18	FAMEs	0.2055	0.183	0.183	0.182		0.0771	0.0762	0.0762	0.0758
		0.1833	0.0170	0.021	0.017		0.0766	0.0013	0.0012	0.0013
		0.1543					0.0749			
		0.1858								
		0.2054								
		0.1652								
19	Free fatty acids	0.42	0.43	0.432	0.45		0.11	0.11	0.1100	0.12
		0.43	nr	0.016			0.11	nr	0.0000	
		0.43					0.11			
		0.41								
		0.45								
		0.45								

		MILK POWDER					INFANT FORMULA			
Lab	Reported as:	Linolenic Acid	Reported mean & exp uncert	SC's calculated mean & sd	As the triglyceride		Linolenic Acid	Reported mean & exp uncert	SC's calculated mean & sd	As the triglyceride
NIST	Triglycerides	0.176	0.185	0.185	0.185		0.098	0.098	0.098	0.098
		0.183	0.009	0.007	0.009		0.098	0.002	0.001	0.002
		0.198					0.099			
		0.184					0.098			
		0.180					0.097			
		0.190					0.100			
		0.175								
		0.182								
		0.197								
		0.182								
		0.179								
		0.189								
23	Triglycerides	0.1533	0.1638	0.1638	0.1638		0.0935	0.0942	0.0942	0.0942
		0.1466	0.0372	0.0208	0.0372		0.0941	0.0072	0.0008	0.0072
		0.1605					0.0950			
		0.1442								
		0.1958								
		0.1821								

		MILK POWDER					INFANT FORMULA			
Lab	Reported as:	Linolenic Acid	Reported mean & exp uncert	SC's calculated mean & sd	As the triglyceride		Linolenic Acid	Reported mean & exp uncert	SC's calculated mean & sd	As the triglyceride
24	Triglycerides	0.1650	0.1596	0.160	0.1596		0.0932	0.0893	0.0893	0.0893
		0.1629	0.0385	0.008	0.0385		0.0801	0.0214	0.0080	0.0214
		0.1671					0.0945			
		0.1629								
		0.1502								
		0.1502								
							Reference value:	0.0982 " 0.0048		

FIGURE CAPTIONS.

Figure 1-6. Plots of participants results for milk powder (top) and a comparison of participants results and the assigned values in SRM 1846 (bottom). Error bars represent expanded uncertainties as reported by the participants, except in the cases labeled "sd," where one standard deviation (either reported by the participant or calculated by the study coordinator, as noted on the individual figures) has been plotted. Dashed lines are drawn across the plots of SRM 1846 data to indicate the boundaries of the expanded uncertainties on the assigned values. Additional information is provided as a caption on the individual figures.



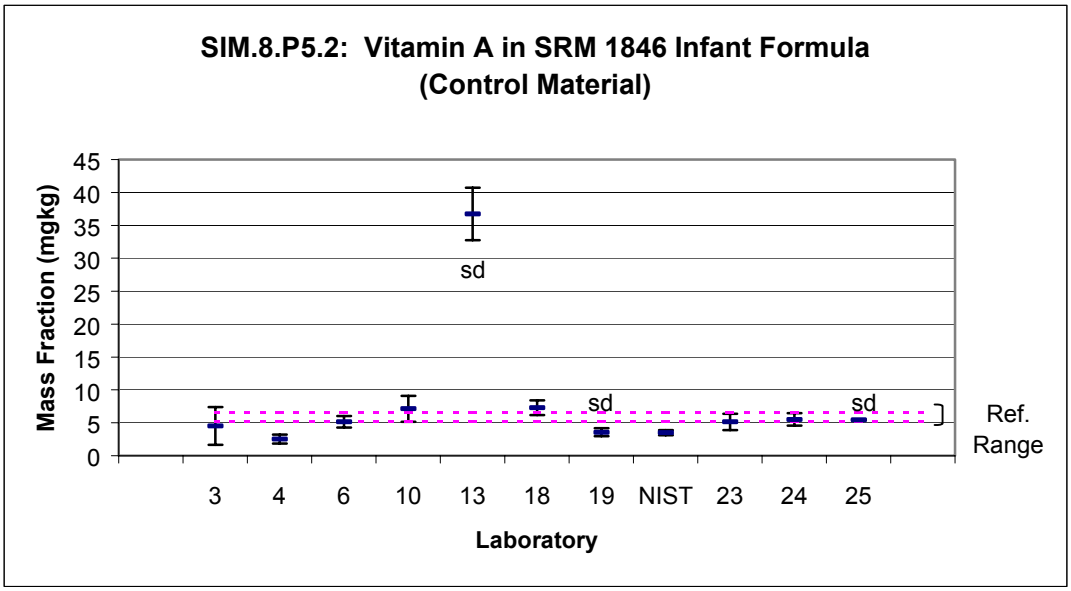


Figure 1. Vitamin A results for test material (milk powder) and control material (SRM 1846). Expanded uncertainties were requested, however some laboratories reported one standard deviation (sd). Standard deviations were calculated by the study coordinator for laboratories 19 and 25 (control material only). The reference range of vitamin A in the control material is indicated by dashed lines. Participants were advised that, depending on their sample preparation procedures, SRM 1846 may not be a useful control material for their vitamin A analyses because the encapsulated vitamin A in the material has become increasingly difficult to extract as the material has aged.

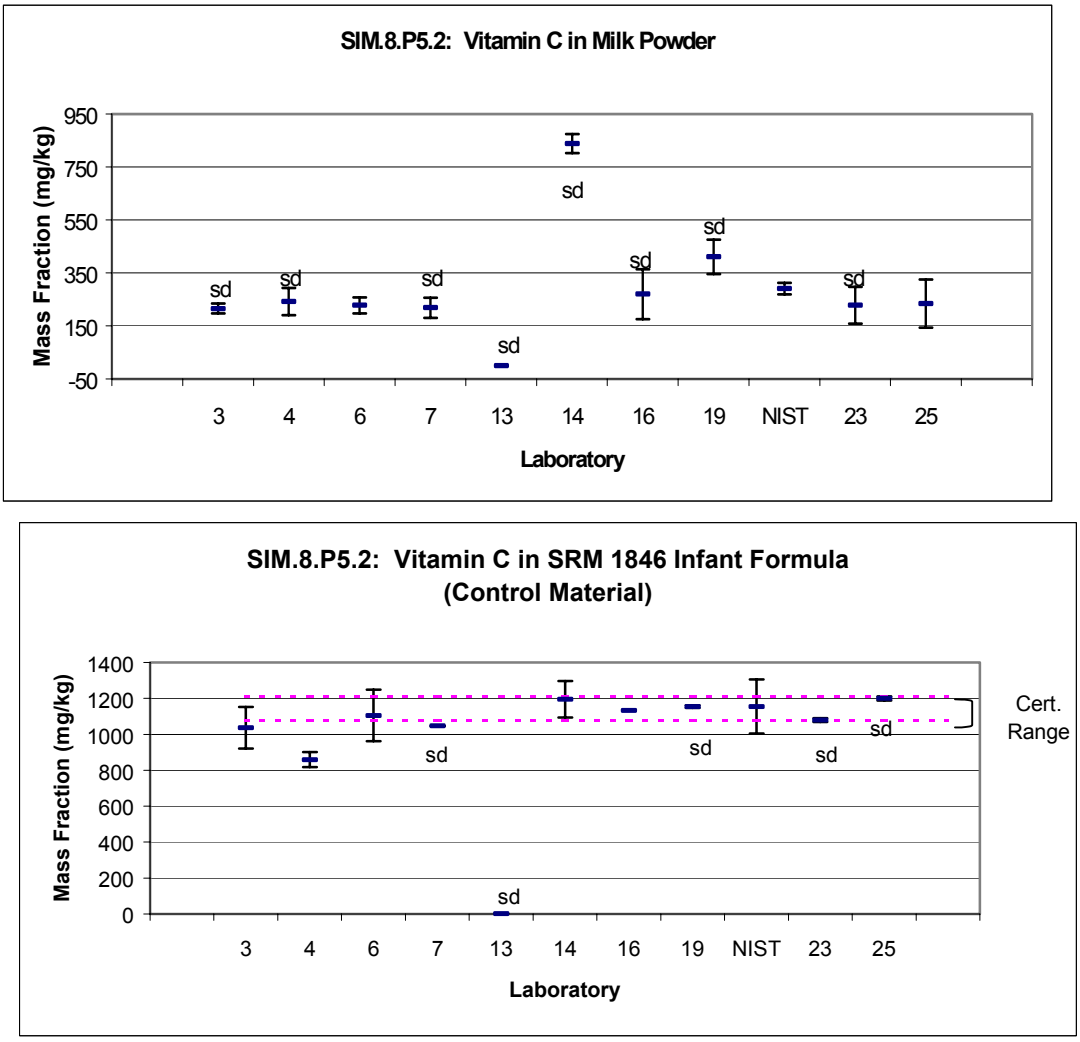
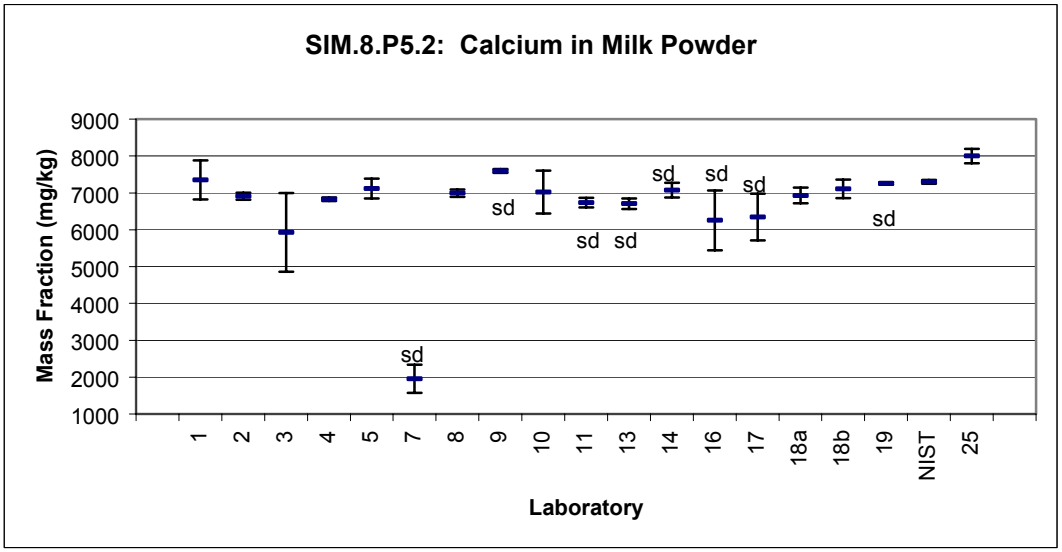


Figure 2. Vitamin C results for test material (milk powder) and control material (SRM 1846). Expanded uncertainties were requested, however some laboratories reported one standard deviation (sd). Means were calculated by the study coordinator for laboratories 7 (test material only), 14 (test material only), 16 (test material only), and 23. Standard deviations were calculated by the study coordinator for laboratories 7 (test material only), 14 (test material only), 16 (test material only), 19, 23, and 25 (control material only). The certified range of vitamin C in the control material is indicated by dashed lines.



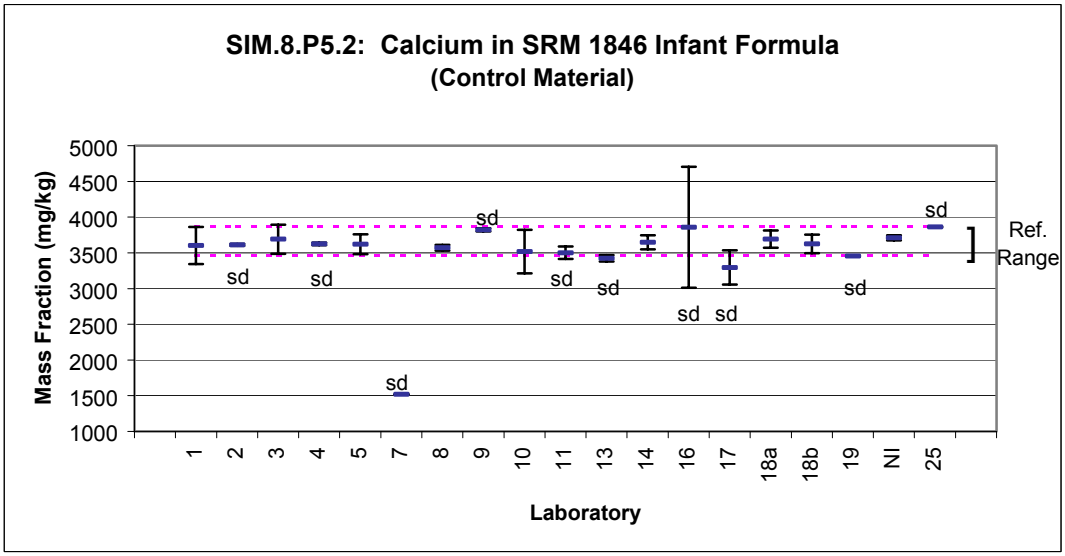


Figure 3. Calcium results for test material (milk powder) and control material (SRM 1846). Expanded uncertainties were requested, however some laboratories reported one standard deviation (sd). Means were calculated by the study coordinator for laboratories 7 (study material only), 14 (study material only), 16 (study material only), and 17. Standard deviations were calculated by the study coordinator for laboratories 2 (control material only), 9, 14 (test material only), 16, 17, 19, and 25 (control material only). The expected range of calcium in the control material is indicated by dashed lines.

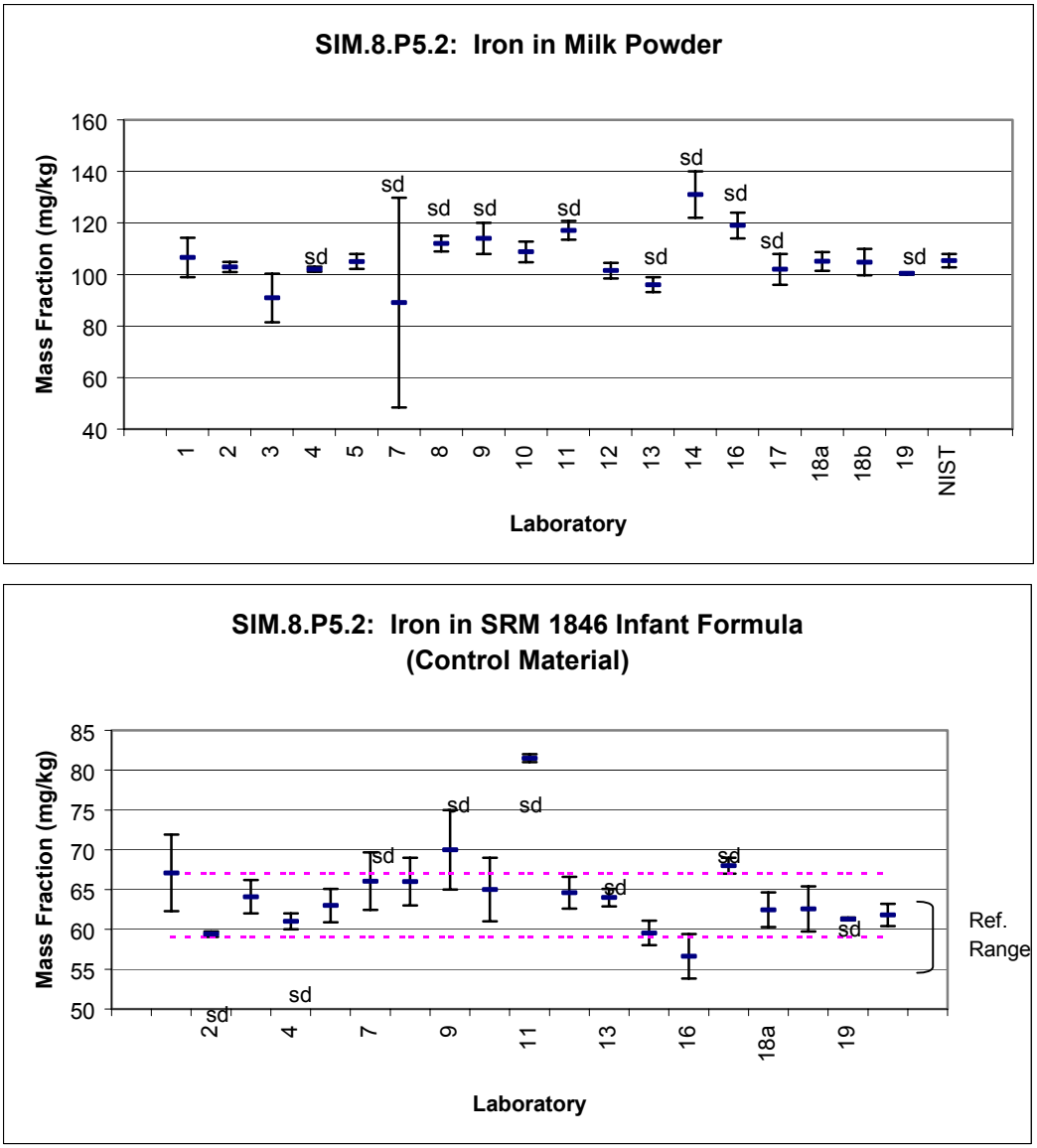
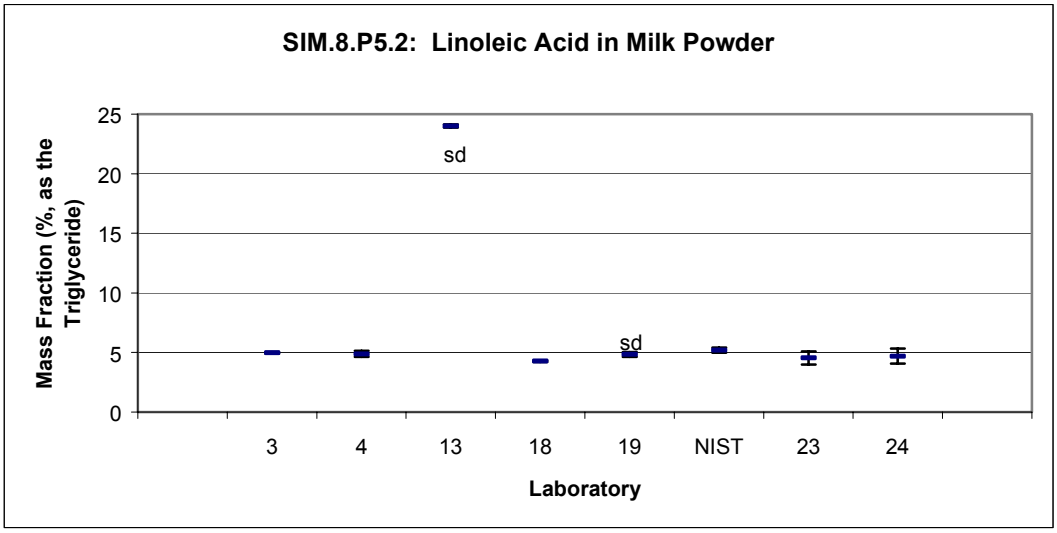


Figure 4. Iron results for test material (milk powder) and control material (SRM 1846). Expanded uncertainties were requested, however some laboratories reported one standard deviation (sd). Means were calculated by the study coordinator for laboratories 7 (test material only), 14 (test material only), 16 (test material only), and 17. Standard deviations were calculated by the study coordinator for laboratories 2 (control material only), 7, 9, 14 (test material only), 16 (test material only), 17, and 19. The expected range of iron in the control material is indicated by dashed lines.



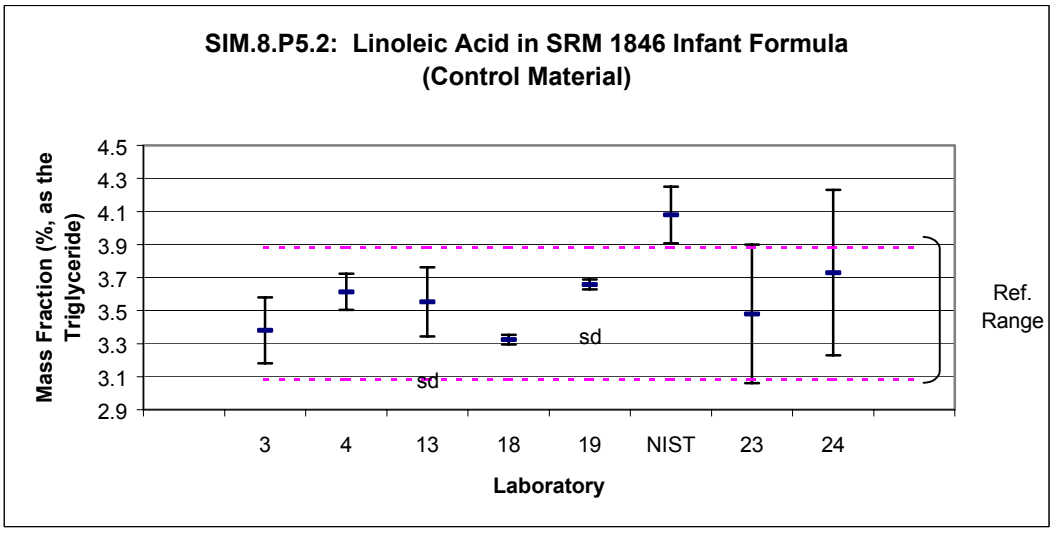


Figure 5. Linoleic acid results for test material (milk powder) and control material (SRM 1846). Expanded uncertainties were requested, however some laboratories reported one standard deviation (sd). Standard deviation was calculated by the study coordinator for laboratory 19. Data reported as free fatty acids or fatty acid methyl esters were converted to triglycerides by the study coordinator. The expected range of linoleic acid in the control material is indicated by dashed lines.

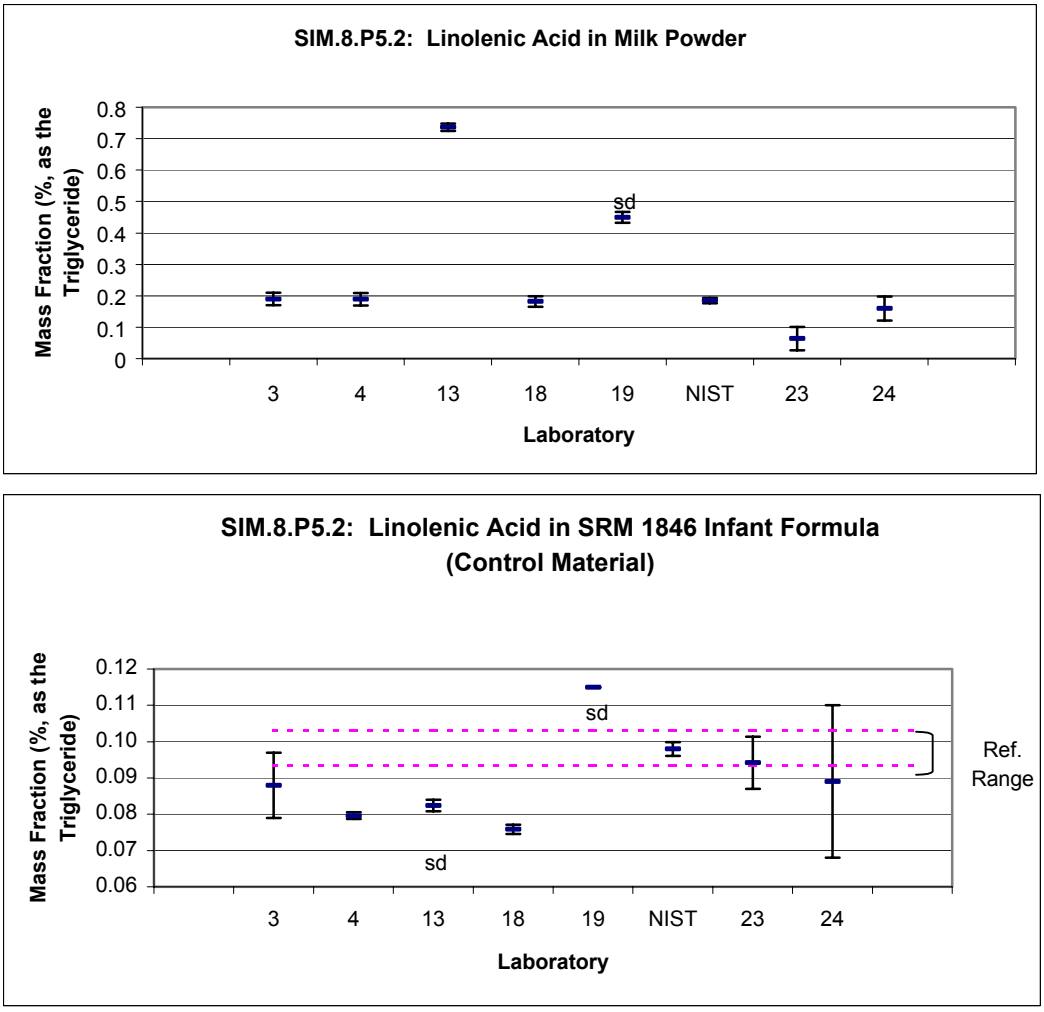


Figure 6. Linolenic acid results for test material (milk powder) and control material (SRM 1846). Expanded uncertainties were requested, however some laboratories reported one standard deviation (sd). Standard deviation was calculated by the study coordinator for laboratory 19. Data reported as free fatty acids or fatty acid methyl esters were converted to triglycerides by the study coordinator. The expected range of linolenic acid in the control material is indicated by dashed lines.

Appendix A. Action plan for infant formula analysis.

I. Project Name: SIM.8.P5.2 Vitamins and Minerals in Infant Formula

II. Dates of Study: October 2001 to July 2002

III. Project Leader: Katherine Sharpless (NIST; 301-975-3121; katherine.sharpless@nist.gov)

IV. Activities: Determination of the concentration of vitamin A, vitamin C, calcium, iron, linoleic acid, and linolenic acid.

V. Expected Outcomes:
   A. Long-term: To determine the measurement capabilities within SIM countries to measure the above-mentioned nutrients in food matrices.
   B. Short-term: Provide a means for participants to compare methods used and results obtained for commonly measured nutrients in a food matrix.

VI. Project Plan:
   A. Analytes – vitamin A, vitamin C, calcium, iron, linoleic acid, linolenic acid. Laboratories will be asked to analyze single portions from each of three packets. A fourth packet will be provided for practice.
   B. Method choice is the decision of the individual laboratory. Each laboratory should provide a complete description of the methods used as well as a description of how the method was calibrated and how the concentrations were calculated. Results should be reported on a mass fraction basis.
   C. Laboratories will need to calculate their uncertainties and provide an explanation of their calculations.
   D. Schedule of activities (target dates)
      Ship materials/instructions to the country coordinators for distribution to participating laboratories (10/01)
      Deadline for receipt of data (4/30/02) *[extension of original deadline]*
      Prepare/distribute report (6/02)

Appendix B. Country coordinators in SIM.8.P5.2 to whom samples and accompanying materials were shipped for distribution to other participants within that country.

Dra. Celia Puglisi
Institute Nacional de Tecnologia Industrial CEFIS
Parque Technologico Miguelete
Av. Gral Paz entre Albarellos y Constituyentes
CC.157
San Martin 1650
Buenos Aires
ARGENTINA
cpuglisi@inti.gov.ar
54-114-752-0818

Sra. Vera M.L. Poncano A. Silva
Institute for Technological Research of São Paulo State
Rua Marques de São Vicente, 225
São Paulo
0-5508-901
BRAZIL
vponcano@ipt.br
55-11-376-74540

Dr. Robert Brousseau
CFIA
1001 St-Laurent West
Longueuil, P. Que J4K 1C7
CANADA
brousseaur@inspection.gc.ca
450-646-7711 x257

Carlos Paniagua
Oficina nacional de normas y unidades de medida (ONNUM)
500 m norte supermercado Munoz y Nanne
Ciudad Cientifica, San Pedro
COSTA RICA
carlos.paniagua@ns.onnum.go.cr

Rafael Lino
Colonia Médica
Ave. Dr. Emilio Alvarez
pje. Dr. Guillermo Rodríguez No. 51
San Salvador
EL SALVADOR
rlino@conacyt.gob.sv
503-225-2608
Ing. Cristina Rodrigues

COHCIT
Col. Alameda
Ave. Julio Lozano Diaz, no 1354
Apartado Postal 4458
Tegucigalpa, MDC
HONDURAS
cristina@cohcit.gob.hn
cristyna6@hotmail.com
504-232-5669

James Kerr
The Bureau of Standards Jamaica
6 Winchester Road
Kingston 10
JAMAICA
jwkerr@hotmail.com
876-926-3140

Alejandro Perez Castorena
Centro Nacional de Metrologia
Km. 4.5 Carretera a los Cues
Municipio el Marqus C.P. 76000
Queretaro Queretaro
MEXICO
52-42-110560

Luis Mussio
Laboratorio Tecnologico del Uruguay
Avenida Italia 6201
CP 11500
Montevideo
URUGUAY
LMUSSIO@latu.org.uy
598-2-601-3724

Katherine Sharpless
National Institute of Standards and Technology
100 Bureau Drive Stop 8392
Gaithersburg, MD 20899-8392
USA
katherine.sharpless@nist.gov
301-975-3121

Appendix C. Letter to country coordinators.

October 2, 2001

Dear Country Coordinator:

Enclosed are the samples for analysis in SIM.8.P5.2, the intercomparison on infant formula/milk powder. I am sending you the required number of samples and data reporting information for distribution to the participants that you previously identified.
Please distribute these samples and the accompanying materials as soon as possible. You will note on the enclosed timeline that data should be reported to me before February 2002.

If you have any questions, I can be reached at 1-301-975-3121 (phone), 1-301-977-0685 (fax), or katherine.sharpless@nist.gov (e-mail).

Sincerely,

Katherine E. Sharpless, Ph.D.
Research Chemist
Analytical Chemistry Division
Chemical Science and Technology Laboratory

Appendix D. Letter to interlaboratory comparison exercise participants and accompanying sheets for reporting results and methodology.

October 15, 2001

Dear Participant:

We are pleased that you have agreed to participate in SIM.QM-P5, the intercomparison of infant formula analyses. Enclosed you will find packets of milk powder (the test sample), Standard Reference Material (SRM) 1846 Infant Formula (for quality control), information and instructions, and forms for reporting your methods and results.

The Certificate of Analysis for SRM 1846 is also enclosed. Please do not use this material to calibrate your instrument. Instead, use it as a control material to determine whether your methods are functioning appropriately. (Because of the difficulty in extracting vitamin A from this material, you are quite likely to obtain a value lower than the reference value when you analyze it, and you are likely to overestimate the content of vitamin A in the test sample if you use the SRM as a calibrant.)

Please return your data and method report forms by February 28, 2002 to:

Katherine Sharpless
NIST
100 Bureau Drive Stop 8392
Gaithersburg, MD 20899-8392
USA
fax: 1-301-977-0685.

If you have any questions about this project, you can contact me at 1-301-975-3121 or katherine.sharpless@nist.gov.

Sincerely,

Katherine E. Sharpless, Ph.D.
Research Chemist
Analytical Chemistry Division
Chemical Science and Technology Laboratory

SIM.8.P5.2
Information and Instructions

Materials

Test Material:
The test material is whole milk powder manufactured by preparing a base of whole milk containing protein, fat, carbohydrate, and minerals, and combining that base with a vitamin premix containing retinyl acetate, dl-alpha-tocopherol acetate, and ascorbate. The packets, which contain approximately 130 g of material, were sealed under a mixture of carbon dioxide and nitrogen.

Control Material:
SRM 1846 Infant Formula is a milk-based infant formula that was manufactured by preparing a spray-dried formula base containing fat, protein, carbohydrate, and minerals, and then combining that formula base with a dry-blend vitamin premix that supplied all of the vitamins. Fat-soluble vitamins were incorporated in the premix in 200-μm cold-water-soluble powders. The final product is composed of 95 % formula base and 5% vitamin premix. The powdered infant formula was sealed under nitrogen in single-use foil packets, each containing approximately 30 g of material.

Storage

Until required for use, the samples should be stored at temperatures between approximately 20 °C and 25 °C in the original, sealed packets. Do not report results for the contents of previously opened packets. If reconstituted, the sample should be used immediately.

Instructions for Use

Before use, the contents of a packet of milk powder should be poured into a larger container (e.g., a large beaker) for mixing. (The packets are too full to allow mixing within the packet itself.) Before use, the contents of a packet of SRM 1846 should be mixed by gently shaking the packet.

Analyses

We have provided you with 12 packets of each material. Please analyze duplicate portions from each of the three packets of milk powder for each set of analytes. Please analyze single portions from each of three packets of SRM 1846 for each set of analytes. Use your laboratory's usual analytical methods.

If you detect both retinol and retinyl acetate (in the milk powder) or retinyl palmitate (in SRM 1846) when you determine vitamin A, please convert your data for the esters and report vitamin A solely as retinol. Indicate whether you measured just *trans*-retinol or total retinol, whether the isomers were resolved, and whether you added them linearly if you reported total retinol.

Because of the difficulty in extracting vitamin A from SRM 1846, you may obtain a vitamin A (retinol) value lower than the reference value. Depending on your method of sample preparation, SRM 1846 *may not* be a useful control material for your vitamin A analysis.

Reporting of Results

Please provide six values for each analyte in the milk powder and three values for each analyte in SRM 1846. Use the enclosed data form. Please report results on an "as-received" basis, not on a dry-mass basis, in the units specified on the report form. Provide methodological information on the forms provided.

Please also provide a brief description of the approach used for calculation of the expanded uncertainty (95% level of confidence), including the standard uncertainty of the individual components. (See the ISO Guide to the Expression of Uncertainty in Measurement or NIST's interpretation of the ISO Guide at http://physics.nist.gov/cuu/Uncertainty/index.html.)

Results are due February 28, 2002. Please return your forms to:

Katherine Sharpless
NIST
100 Bureau Drive, MS 8392
Gaithersburg, MD 20899-8392
USA
fax: 1-301-977-0685.

Information or questions may be e-mailed to katherine.sharpless@nist.gov.

VITAMIN A

Brief description of sample preparation procedures (including sample size, internal standard, saponification conditions, extracting solvent, etc.)

Brief description of chromatographic method (including analytical column, mobile phase, detector, wavelengths, etc.)

Brief description of uncertainty analysis (including source of uncertainty and its standard uncertainty, degrees of freedom, type A or B, value of k, source of data [e.g., historical, calibration certificate], etc.)

VITAMIN C

Brief description of sample preparation procedures (including sample size, internal standard, saponification conditions, extracting solvent, etc.)

Brief description of chromatographic method (including analytical column, mobile phase, detector, wavelengths, etc.)

Brief description of uncertainty analysis (including source of uncertainty and its standard uncertainty, degrees of freedom, type A or B, value of k, source of data [e.g., historical, calibration certificate], etc.)

LINOLEIC ACID AND LINOLENIC ACID

Brief description of sample preparation procedures (including sample size, internal standard, hydrolysis conditions, etc.)

Brief description of chromatographic method (including analytical column, detector, etc.)

Brief description of uncertainty analysis (including source of uncertainty and its standard uncertainty, degrees of freedom, type A or B, value of k, source of data [e.g., historical, calibration certificate], etc.)

CALCIUM

Brief description of sample preparation procedures (including sample size, digestion conditions, etc.)

Brief description of spectrometric method (including instrumentation, wavelengths, etc.)

Brief description of uncertainty analysis (including source of uncertainty and its standard uncertainty, degrees of freedom, type A or B, value of k, source of data [e.g., historical, calibration certificate], etc.)

IRON

Brief description of sample preparation procedures (including sample size, digestion conditions, etc.)

Brief description of spectrometric method (including instrumentation, wavelengths, etc.)

Brief description of uncertainty analysis (including source of uncertainty and its standard uncertainty, degrees of freedom, type A or B, value of k, source of data [e.g., historical, calibration certificate], etc.)

Appendix E. Methods used for analysis and calculation of uncertainties.

Intercomparison of Infant Formula Analyses
SIM.QM-P5

LABORATORY:

IRON

Brief description of sample preparation procedures (including sample size, digestion conditions, etc.)

Samples and reference (≈3 g) were dried on platinum capsule, flamed to carbonize and calcinated in muffle furnace during 3 h at 600ºC. Residues were dissolved in HCl 2 M hot solution and diluted to 50,0 ml in volumetric flask with the same dissolvent after cooling.
Posterior dilutions were made in order to achieve the linear range for AAS measurements.

Brief description of spectrometric method (including instrumentation, wavelengths, etc).

Instrument: Perkin Elmer Analyst 100 atomic absorption spectrometer.
Wavelength: 248,3 nm
Slitwidth: 0,2 nm
Flame: Air – Acetylene
Calibration: Standard Additions (4 points). Linear interception.
Standard Solutions: from Titrisol Merck (Fe<III>), 1,000 g/L.

Brief description of uncertainty analysis (including source of uncertainty and its standard uncertainty, degrees of freedom, type A or B, value of k, source of data [e.g. historical, calibration certificate], etc).

Sources of uncertainty
Weighing: 0,014 %
Dilutions + additions: 2,1 %
Measurement (signal fluctuation + linear regression) = 1,5 % k=2
Expanded uncertainty (A+B,k) = 7,2%

CALCIUM

Brief description of sample preparation procedures (including sample size, digestion conditions, etc.)

See IRON.

Brief description of spectrometric method (including instrumentation, wavelengths, etc).

Instrument: Perkin Elmer Analyst 100, flame atomic absorption spectrometer
Wavelength: 422,7 nm
Slitwidth: 0,70 nm
Flame: Air – Acetylene
Calibration: Standard Additions (4 points)
Standard Solutions: from Titrisol Merck (Ca<II>), 1,000 g/L.

Brief description of uncertainty analysis (including source of uncertainty and its standard uncertainty, degrees of freedom, type A or B, value of k, source of data [e.g. historical, calibration certificate], etc).

See IRON.

Intercomparison of Infant Formula Analyses
SIM.QM-P5

CALCIUM

Brief description of sample preparation procedures (including sample size, digestion conditions, etc.)

We take 0,80g of sample. We made ash to 550°C, we diluted the ashes in HNO_3 to produce a final concentration of 4 % M/V HNO_3. The final volume was 100 mL.

Brief description of spectrometric method (including instrumentation, wavelengths, etc.)

The method was ICP-OES. The wavelength was 317.933 nm. We use six calibration standards of 10, 20, 30, 40, 50 and 60 µg/mL. We applied weighted regression linear.

Brief description of uncertainty analysis (including source of uncertainty and its standard uncertainty, degrees of freedom, type A or B, value of k, source of data [e.g., historical, calibration certificate], etc.)

The sources of uncertainty were.

a) The balance with an uncertainty of ± 0,15 mg for the calibration / linearity contribution.

b) The volumetric flask with an uncertainty of ± 0,10 mL for the calibration (triangular distribution), temperature ± 3°C (rectangular distribution).

c) The weighted linear regression with an uncertainty of 0,3 mg/L

K was taken as 2

The source of data was in some cases calibration certificate and sometimes historical (regression)

Intercomparison of Infant Formula Analyses
SIM.QM-P5

IRON

Brief description of sample preparation procedures (including sample size, digestion conditions, etc.)

We take 0,8 g of sample. We made ash to 550°C, we diluted the ashes in HNO_3 to produce a final concentration of 4% v/v HNO_3. The final volume was 100 mL.

Brief description of spectrometric method (including instrumentation, wavelengths, etc.)

The method was ICP-OES. The wavelength wes 238.204 nm
We use six standards calibration of 0.15; 0,20; 0,40; 0,60; 0,80 and 1,00 mg/L. We applied weighted regression linear

Brief description of uncertainty analysis (including source of uncertainty and its standard uncertainty, degrees of freedom, type A or B, value of k, source of data [e.g., historical, calibration certificate], etc.)

I done calcium except

c) The weighted linear regression had an uncertainty of 0,007 mg/L

K was taken as 2

The source of data was the same as in calcium.

Intercomparison of Infant Formula Analyses
SIM.QM-P5

LINOLEIC ACID AND LINOLENIC ACID

Brief description of sample preparation procedures (including sample size, internal standard, hydrolysis conditions, etc.)

- Dissolve 10g of sample in 200 mL of ethyl ether.
- Set the pH 2.0 with concentrated HCl.
- Stir the solution for 1 hour.
- Filter the solution through Na_2SO_4 anidrous.
- Evaporate the solvent at room temperature.
- The fat extracted was used to proper the fatty acids methyl esters (FAMEs).
- About 200 mg of fat was esterified to FAMEs following IUPAC method 2301.

Brief description of chromatographic method (including analytical column, detector, etc.)

- GC column - 100% biscyanopropyl polysiloxane (SP- 2340).
Column length - 60 m.
Film thickness - 0.20 um.
- Injection volume - 1 uL.
- Injector temperature - 220 C.
- Detector temperature - 220 C.
- Programmed oven temperature: 60C (2min) --15C/min--135C(1min)-- -- 3C/min -- 215C (5min).
- Detector - FID.
- Calculation - Normalisation -(% FAME)X(0.952) -- Expres triglicerides

Brief description of uncertainty analysis (including source of uncertainty and its standard uncertainty, degrees of freedom, type A or B, value of k, source of data [e.g., historical, calibration certificate], etc.)

- Evaluation of standard uncertainty - TYPE A
- Degrees of freedon - N - 1 = 5.
- Value of (v) from t- distribution (p= 95%) - 2.57.
- u = the estimated standard deviation of the mean =

$$2.57 \frac{\sigma}{\sqrt{N}}$$

σ = standard deviation
N = 6

Intercomparison of Infant Formula Analyses SRM 1846 Infant formula

Calcium

Brief description of sample preparation procedures (including sample size, digestion conditions, etc.)

- Dry-ashing at 450°C
- Ashing-aid: HNO_3
- Sample size(g): 0.5000
- Final volume (mL): 50
- Calibration curve: 2.50 to 100.0 mg/L

Brief description of spectrometric method (including instrumentation, wavelengths, etc.)

- Inductively coupled plasma atomic emission spectrometry (ICP-AES)

Perkin-Elmer- OPTIMA 3000DV

RF power: 1350 W

Nebulizer flow (L. min^{-1}):0.85

Auxiliary flow (L. min^{-1}): 0.5

Plasma flow (L. min^{-1}): 15

Sample flow (mL. min^{-1}): 1.0

- View plasma: radial
- Observation height: 15 mm above load coil
- Nebulizer: gem tip (cross-flow)
- Spray chamber: Scott type
- Wavelength: 422.673 nm
- Standard torch
- Auto integration time: min: 1 s max: 5s
- Number of replicates: 3
- Background correction: manual selection of points
- Measurement processing mode: area

Intercomparison of Infant Formula Analyses SRM 1846 Infant formula

IRON

Brief description of sample preparation procedures (including sample size, digestion conditions, etc.)

- Dry-ashing at 450°C
- Ashing-aid: HNO_3
- Sample size(g): 2.0000
- Final volume (mL): 10
- Calibration curve: 0.25 to 10.0 mg/L

Brief description of spectrometric method (including instrumentation, wavelengths, etc.)

- Inductively coupled plasma atomic emission spectrometry (ICP-AES)

Perkin-Elmer- OPTIMA 3000DV

RF power: 1350 W

Nebulizer flow (L. min^{-1}):0.85

Auxiliary flow (L. min^{-1}): 0.5

Plasma flow (L. min^{-1}): 15

Sample flow (mL. min^{-1}): 1.0

- View plasma: axial
- Nebulizer: gem tip (cross-flow)
- Spray chamber: Scott type

- Nebulizer: gem tip (cross-flow)
- Spray chamber: Scott type
- Wavelength: 259.940 nm
- Standard torch
- Auto integration time: min: 1 s max: 5s
- Number of replicates: 3

Intercomparison of Infant Formula Analyses SRM 1846 Infant formula

Calcium

Brief description of uncertainty analysis (including source of uncertainnty and its standard uncertainty, degrees of freedom, type A or B, value of k, source of data (e.g., historical, calibration certificate), etc.)

Evaluation of uncertainty: Type A

Degrees of freedom: $\nu = n-1$

U: Expanded uncertainty

$U = K.u$

$U = 2 \times 102 = 204$

u: Combined standard uncertainty

$u = t.s/n^{1/2}$

$u = 2.52 \times 70.2/3^{1/2} = 102$

K: Coverage factor used to calculate expanded uncertainty

K=2 (considering confidence level of approximately 95%)

s: Standard deviation of the sample

s = 70.2

$t_{95.45\%,\,6} = 2.52$

IRON

Brief description of uncertainty analysis (including source of uncertainnty and its standard uncertainty, degrees of freedom, type A or B, value of k, source of data (e.g., historical, calibration certificate), etc.)

Evaluation of uncertainty: Type A

Degrees of freedom: $\nu = n-1$

U: Expanded uncertainty

$U = K.u$

$U = 2 \times 1.05 = 2.1$

u: Combined standard uncertainty

$u = t.s/n^{1/2}$

$u = 2.52 \times 0.72/3^{1/2} = 1.05$

K: Coverage factor used to calculate expanded uncertainty

K=2 (considering confidence level of approximately 95%)

s: Standard deviation of the sample

$s = 0.72$

$t_{95.45\%,\,6} = 2.52$

Intercomparison of Infant Formula Analyses
SIM.QM-P5

VITAMIN C

Brief description of sample preparation procedures (including sample size, internal standard, saponification conditions, extracting solvent, etc.)

- ✓ Cupric ions, reduction
- ✓ Spectrophotometric
- ✓ Sample size (g): 2
- ✓ Extracting solvent: *meta*-Phosphoric Acid 5%+ H2SO4 0,1 N
- ✓ Complexant: Isoamyl alcohol + toluene + Cupric Acetate and Cuproine
- ✓ Wavelenghts: 545 nm
- ✓ Standard: Ascorbic Acid 99,7%

Brief description of chromatographic method (including analytical column, mobile phase, detector, wavelengths, etc.)

- ✓ We did not use chromatographic method

Brief description of uncertainly analysis (including source of uncertainty and its standard uncertainty, degrees of freedom, type A or B, value of k, source of data (e.g. historical, calibration certificate) etc.

- ✓ Guidelines for evaluating and expressing the uncertainty of NIST measurement results
- ✓ type A
- ✓ degrees of freedom ==> $t_{N-1\ (p=95\%)}$
- ✓ s=estimated standard deviation
- ✓ $u(x_i)=s/(N)^{1/2}$
- ✓ $u_t= t_{N-1} * u(x_i) = t_{N-1\ (p=95\%)} * s/(N)^{1/2}$

Intercomparison of Infant Formula Analyses
SIM.QM-P5

VITAMIN A

Brief description of sample preparation procedures (including sample size, internal standard, saponification conditions, extracting solvent, etc.)

- ✓ Methods of analysis-AOAC,1990 – 15th,pag.1045-1047
- ✓ Sample size (g): 3
- ✓ Extraction procedure: Alkaline Hydrolysis (saponification)
- ✓ Extraction Solvente: petroleum Ether
- ✓ Spectrophotometric: SbCl3 reagent
- ✓ Walenghts: 620 nm

Brief description of chromatographic method (including analytical column, mobile phase, detector, wavelengths, etc.)

- ✓ We did not use chromatographic method

Brief description of uncertainly analysis (including source of uncertainty and its standard uncertainty, degrees of freedom, type A or B, value of k, source of data (e.g. historical, calibration certificate) etc.

- ✓ Guidelines for evaluating and expressing the uncertainty of NIST measurement results
- ✓ type A
- ✓ degrees of freedom ==> $t_{N-1\ (p=95\%)}$
- ✓ s=estimated standard deviation
- ✓ $u(x_i)=s/(N)^{1/2}$
- ✓ $u_t= t_{N-1} * u(x_i) = t_{N-1\ (p=95\%)} * s/(N)^{1/2}$

Intercomparison of Infant Formula Analyses
SIM.QM-P5

LINOLEIC ACID AND LINOLENIC ACID

Brief description of sample preparation procedures (including sample size, digestion conditions, etc.)

3g of sample was weighted, hidrolysed with 60 mL hydrocloric acid 37% plus 100 mL of water (40 min/100°C) and filtered through filter paper. The acid was removed from the paper by water wash and then dried in 100 °C oven, and the fat extracted through 6 h reflux with petroleum ether in a *Butt* extractor. The solvent was evaporated and the fat weighted. After total fat quantification 0,3 g of lipidic material was transferred to an appropriate vial with screw cap, and saponified with 5 mL of methanolic sodium hydroxide for 15 min/65°C. The methyl esters were obtained by addition of 10 mL of ammonium chloride-methanol-sulfuric acid (2g/60 mL/3mL) solution, keeping in a hot bath (60°C) by 10 min. The vial was cooled, and a saturated solution of sodium cloride was added and mixed. The petroleum ether layer was separated and injected in GC-FID. The calculation was made by multiplying the area of linoleic or linolenic fatty acid methyl ester in normalized chromatogram by total fat content.
Reference: HARTMAN, L.; LAGO, R.C.A. Rapid preparation of fatty acid methyl esters from lipids. Lab. Practice, v.22, n.8, p.475-476, 1973.

Brief descrition of spectrometric method (including instrumentarion, wavelengths, etc.)
Chromatographic conditions:
Equipment: Konic model HRGC 4000A Gas chromatograph, with flame ionization detector.
Column:
CP Sil 88 Tailor Made FAME(Chrompak) 50mx0,25 mm id, film thickness 0,2 µm; Carrier: Hidrogen;Flux: 0,5 mL/min
Oven:
Initial: 180°C/2 min
Rate: 5 °C/min
Final temperature 225 °C/15 min
Detector:
Temperature: 300 °C
Make-up Nitrogen: 30 mL/min
Syntetic Air: 220 mL/min
Hydrogen 38mL/min
Injector: temperature 270 °C;split ratio: 1:75
Injection volume: 0,5 µL

Brief descrition of uncertainty analysis (including source of uncertainty and its standard uncertainty, degrees of freedom, type A or B, value of k, source of data [eg. Historical, calibration cerificate], etc.)

Source	Uncertainty	Source of data	k	Type
Balance	± 0,40mg	Calibration certificate	2	B
Sample mean and standard desviation				A

CALCIUM

Brief description of sample preparation procedures (including sample size, digestion conditions, etc.)

Mineralization of mineral elements: 500mg of sample in furnace were mineralized at 450°C for 16 h, using the method of AOAC (1998). The ash was dissolved in 1.25 mL of concentrated nitric acid and diluted to 25 mL with deionized water.

Brief descrition of spectrometric method (including instrumentarion, wavelengths, etc.)

Equipament: Inductively coupled plasma atomic emission (ICP-AES), model 2.000 BAIRD, simultaneous version.
The ICP conditions were:
⇒ radio frequency generator 40 MHz;
⇒ power 1000 W;
⇒ nebulizator pneumatic concentric;
⇒ torch conventional (low flow);
⇒ flow rate gas in the plasma = 14 L/min; ⇒ flow rate argon auxiliary = 0.7 L/min;
⇒ flow rate argon of refrigeration = 0.7 L/min;
⇒ flow rate sample 2.1 mL/min.
Lines of emission: Calcium → 317.93nm

Brief descrition of uncertainty analysis (including source of uncertainty and its standard uncertainty, degrees of freedom, type A or B, value of k, source of data [eg. Historical, calibration cerificate], etc.)

Source	Uncertainty	Source of data	Valid until	Type
Balance	± 0,40mg	Calibration certificate	20/12/02	B
Pipette (HandyStep Eletronic)	± 0,06mg	Calibration certificate	10/09/02	B
Volumetric flask 25mL	± 0,04mL	Calibration certificate	---	B
Volumetric flask 50mL	± 0,06mL	Calibration certificate	---	B
Volumetric flask 100mL	± 0,08mL	Calibration certificate	----	B
Solution standard – Ca (1000mg)	± 2mg	Calibration certificate	11/2004	B
Solution standard – Fe (1000mg)	± 3mg	Calibration certificate	09/2003	B
Sample mean and standard desviation				A
Standard calibration				A

K = 2

IRON

Brief description of sample preparation procedures (including sample size, digestion conditions, etc.)

Mineralization of mineral elements: 500mg of sample in furnace were mineralized at 450°C for 16 h, using the method of AOAC (1998). The ash was dissolved for 2,5 mL of concentrated nitric acid and diluted to 50 mL with deionized water.

Brief descrition of spectrometric method (including instrumentarion, wavelengths, etc.)

Equipament: Inductively coupled plasma atomic emission (ICP-AES), model 2.000 BAIRD, simultaneous version.
The ICP conditions were:
⇒ radio frequency generator 40 MHz;
⇒ power 1000 W;
⇒ nebulizator pneumatic concentric;
⇒ torch conventional (low flow);
⇒ flow rate gas in the plasma = 14 L/min; ⇒ flow rate argon auxiliary = 0,7 L/min;
⇒ flow rate argon of refrigeration = 0,7 L/min;
⇒ flow rate sample 2,1 mL/min.
Lines of emission: Iron → 259,94nm

Brief descrition of uncertainty analysis (including source of uncertainty and its standard uncertainty, degrees of freedom, type A or B, value of k, source of data [eg. Historical, calibration cerificate], etc.)

Source	Uncertainty	Source of data	Valid until	Type
Balance	± 0,40mg	Calibration certificate	20/12/02	B
Pipette (HandyStep Eletronic)	± 0,06mg	Calibration certificate	10/09/02	B
Volumetric flask 25mL	± 0,04mL	Calibration certificate	---	B
Volumetric flask 50mL	± 0,06mL	Calibration certificate	---	B
Volumetric flask 100mL	± 0,08mL	Calibration certificate	----	B
Solution standard – Ca (1000mg)	± 2mg	Calibration certificate	11/2004	B
Solution standard – Fe (1000mg)	± 3mg	Calibration certificate	09/2003	B
Sample mean and standard desviation				A
Standard calibration				A

K = 2

Intercomparison of Infant Formula Analyses
SIM.QM-P5

VITAMIN C

Brief description of sample preparation procedures (including sample size, internal standard, saponification conditions, extracting solvent, etc.)

The sample were completely mixed and 1,0g samples were taken for analysis. The vitamin were extracted with 3% meta-phosphoric acid in 8% acetic acid solution. Ascorbic acid were oxided to dehydroascorbic acid with Norit, followed by reaction with o-phenylenediamine to form a fluorescence derivative. The fluorescence derivative were separated and detected in chromatographic system with fluorescence detector.

Brief description of chromatographic method (including analytical column, mobile phase, detector, wavelengths, etc.)

Chromatographic system:

- Column: C18 125mmx4mm (5□m);
- Mobile phase: Methanol: H_2O (55:45), isocratic;
- Flow rate: 0,5 ml/min.;
- Temperature: ambient (20±5°C);
- Injection volume: 200□l
- Detector: fluorescence detector (□ $_{Excitation}$ = 350nm, □$_{Emission}$ = 430nm).
- Retention times: about 6 minutes.

Brief description of uncertainty analysis (including source of uncertainty and its standard uncertainty, degrees of freedom, type A or B, value of k, source of data [e.g., historical, calibration certificate], etc.)

Standard uncertainty:

Source	Uncertainty	Source of data	Type
Balance	± 0.060mg	calibration certificate	B
Pipette	± 16.33□l	calibration certificate	B
Volumetric flask 10ml	± 0.04ml	calibration certificate	B
Volumetric flask 25ml	± 0.04ml	calibration certificate	B
Volumetric flask 50ml	± 0.06ml	calibration certificate	B
Volumetric flask 100ml	± 0.1ml	calibration certificate	B

Vitamin C standard (external calibration) = L (+) Ascorbic acid GR for analysis and for biochemistry ACS, ISO - (Merck 1.00127.0100, Lot F854227).

Intercomparison of Infant Formula Analyses
SIM.QM-P5

VITAMIN A

Brief description of sample preparation procedures (including sample size, internal standard, saponification conditions, extracting solvent, etc.)

The samples were completely mixed and 2.5g samples were taken for analysis. The samples were saponified for 30 minutes at 80°C to 90°C with a 50% (mass/volume) potassium hydroxide solution. The analytes were extracted into diethyl ether which was evapored. The analytes were redissolved in n-hexane and injected onto a chromatographic system.

Brief description of chromatographic method (including analytical column, mobile phase, detector, wavelengths, etc.)

Chromatographic system:

- Column: Si60 125mmx4mm (5□m);
- Mobile phase: n-hexane : isopropanol (98:2), isocratic;
- Flow rate: 1,5 ml/min.;
- Temperature: 40°C;
- Injection volume: 100□l
- Detector: fluorescence detector (□ $_{Excitation}$ = 325nm, □$_{Emission}$ = 480nm).
- Retention times: about 5 minutes.

Brief description of uncertainty analysis (including source of uncertainty and its standard uncertainty, degrees of freedom, type A or B, value of k, source of data [e.g., historical, calibration certificate], etc.)

Standard uncertainty:

Source	Uncertainty	Source of data	Type
Balance	± 0.40mg	calibration certificate	B
Pipette	± 6.07□l	calibration certificate	B
Spectrometer	± 0.014 Abs	calibration certificate	B
Volumetric flask 10ml	± 0.04ml	calibration certificate	B
Volumetric flask 25ml	± 0.04ml	calibration certificate	B
Volumetric flask 50ml	± 0.06ml	calibration certificate	B
Volumetric flask 100ml	± 0.1ml	calibration certificate	B

Vitamin A standard (external calibration) = *all* trans-retinol (Sigma R-7632, Lot 110k5001)
The concentration of the standard were rectified after spectrophotometric analysis ($E^{1\%}_{1cm}$ = 1835 - □ = 325nm).

Intercomparison of Infant Formula Analyses
SIM.QM-P5

Calcium and iron

Brief description of sample preparation procedures (including sample size, digestion conditions, etc.)

For both samples and reference materials, portions of about 250 mg were direct weighed into high-purity polyethylene vials, made by Vrije Universiteit, Amsterdam, The Netherlands. Considering that the results are to be reported for samples as received, procedures for moisture correction were adopted only for internal quality control materials. NiCr neutron flux monitor wires, with homogeneity better than 0.35% for Cr, were used at the top and bottom of each vial. Vials were arranged together, wrapped with aluminum foils and inserted into aluminum rabbits (20x70 mm). The material was irradiated in the nuclear research reactor IEA-R1m at IPEN/CNEN, São Paulo, for 8 hours with a thermal neutron flux ranging between 1.0 and 1.4×10^{13} cm^{-2} s^{-1}, depending on the sample position.

Brief description of spectrometric method (including instrumentation, wavelengths, etc.)

The induced gamma radioactivity was measured three times with germanium detectors of 45 and 50% relative efficiency (for ^{60}Co), after cooling periods of about 8, 10 and 18 days. Sample-to-detector distances were 13, 2.5 and 2.3 cm respectively for the three measurements. Spectrum analysis was carried out using the in-house software QuantuMCA, and the concentrations were determined by the k_0-method, using the in-house software Quantu-INAA. The k_0 parameters suggested by De Corte (1987) were adopted. Calcium concentrations were obtained from both 159 and 1296 keV lines, and iron from 1099 and 1292 keV peaks.

Brief description of uncertainty analysis (including source of uncertainty and its standard uncertainty, degrees of freedom, type A or B, value of k, source of data [e.g., historical, calibration certificate], etc.)

Uncertainty budget attached

De Corte, F. The k_0-standardization method – a move to the optimization of neutron activation analysis, Rijksuniversiteit, Gent, 1987.

UNCERTAINTY BUDGET relative standard uncertainties (%) for the laboratory means						
Source	**Evaluation**	**Type A/B**	**Milk Powder**		**SRM1846**	
			Ca	**Fe**	**Ca**	**Fe**
weighing precision	replicated measurements	A	<0.1	<0.1	<0.1	<0.1
weighing accuracy	from calibration certificate	B	<0.1	<0.1	<0.1	<0.1
thermal flux gradient	estimated from sample geometry and vertical flux gradient	B	0.2	0.2	0.2	0.2
flux monitor	systematic uncertainty on flux composition	B	0.3	0.3	0.3	0.3
epithermal flux	derived from determination of flux ratio and alpha parameter	B	0.2	0.1	0.2	0.1
counting efficiency	estimated from efficiency calibration	B	0.4	0.3	0.4	0.3
sample position	possibility of incorrect sample-to-detector distance	B	0.7	0.5	0.7	0.5
sample geometry	filling height measurement	A	0.4	0.3	0.4	0.3
counting statistics	square root of counts for all peaks from all measurements	B	0.1	0.8	0.2	1.0
peak fitting	from least square fitting (average)	A	0.5	0.2	0.5	0.2
k_0 parameters	from literature (De Corte, 1987)	B	0.8	0.3	0.8	0.3
Combined uncertainties (%)			**1.9**	**1.4**	**1.9**	**1.7**
Expanded uncertainties (k=2)			**3.8**	**2.8**	**3.8**	**3.4**

Intercomparison of Infant Formula Analyses
SIM.QM-P5

VITAMIN A

Brief description of sample preparation procedures (including sample size, internal standard, saponification conditions, extracting solvent, etc.)

30 and 60 g of powder (Infant Formula and milk powder) are dissolved in hot water. Immediately after dissolution, 2% ethanolic pyrogallol is added rapidly, following of 10% KOH and finally 2% ethanolic pyrogallol. The extract were stirred for 18 hours at room temperature.

An aliquot of the digest is extracted with a mixture of diethyl ether and hexane in a centrifuge tube. Hexadecane is added to the extract to prevent destruction of the vitamin after evaporation. The residue is dissolved in heptane and chromatographed on a silica column.

Brief description of chromatographic method (including analytical column, mobile phase, detector, wavelengths, etc.)

All-trans retinol and 13-cis retinol are resolved on a 15 cm X 4.5 mm column of Silica (Jones Chromatography). The detection of each isomer is done with a Photodiode Array detector at 325 nm. Quantitation of all-trans retinol and 13-cis retinol was done by external standard. Total vitamin A = sum of all-trans retinol and 13-cis retinol.

Brief description of uncertainty analysis (including source of uncertainty and its standard uncertainty, degrees of freedom, type A or B, value of k, source of data [e.g., historical, calibration certificate], etc.)

Our uncertainty analysis is based on a precision and a bias study. We completed the measurement of uncertainty with the identification of other uncertainty contributions not adequately covered by the precision and bias studies and evaluation of the other uncertainty contributions.
We did a type A evaluation of Standard Uncertainty by calculating the standard deviation of the mean of a series of independent observations. Our degrees of freedom was 36.
Then we estimated the combined standard uncertainty "μ_c" using the usual "root-sum-of-squares" method.
Finally to evaluate the expanded uncertainty "*U*" we used a coverage factor (*k*) of 2.03 giving a level of confidence of approximately 95 percent.

VITAMIN C

Brief description of sample preparation procedures (including sample size, internal standard, saponification conditions, extracting solvent, etc.)

10 g of Infant formula and milk powder were homogeneized in a 250 or 500 ml stoppered volumetric flask in enough cold 17% metaphosphoric acid to give a final concentration of 0.85% (v/v).Homogenates were submitted to 11500 RFC in a refrigerated centrifuge for 15 minutes at 8°C. The clear supernatant was used to estimate Total Vitamin C (Ascorbic Acid (AA) + Dehydroascorbic Acid (DHAA)) in presence of DL-Homocysteine at pH 7.0-7.2 for 30 minutes at 25°C. Quantification was done by external standard with a standard curve in the range of 0.5 to 3.0 ng/20µL.

Brief description of chromatographic method (including analytical column, mobile phase, detector, wavelengths, etc.)

Ascorbic Acid is separated on C_{18} reverse-phase radial pak . The mobile phase is composed of 80 mM sodium acetate, containing 1mM n-Octylamine, 10% methanol and 0.015% metaphosphoric acid. The final pH of the mobile phase is 4.6 and the flow rate is 0.9 ml/min. Automatic injector with cooling. The detection is done with an amperometric detector with glassy carbon electrode (potential, + 0.7V vs Ag/AgCl (20 nA).

Brief description of uncertainty analysis (including source of uncertainty and its standard uncertainty, degrees of freedom, type A or B, value of k, source of data [e.g., historical, calibration certificate], etc.)

Our uncertainty analysis is based on a precision and a bias study. We completed the measurement of uncertainty with the identification of other uncertainty contributions not adequately covered by the precision and bias studies and evaluation of the other uncertainty contributions. We did a type A evaluation of Standard Uncertainty by calculating the standard deviation of the mean of a series of independent observations. Our degrees of freedom was 20. Then we estimated the combined standard uncertainty "μ_c" using the usual "root-sum-of-squares" method. Finally to evaluate the expanded uncertainty "*U*" we used a coverage factor (*k*) of 2.09 giving a level of confidence of 95 percent.

Intercomparison of Infant Formula Analyses
SIM.QM-P5

Brief description of the sample preparation process for the analysis of iron, calcium.

Weight 2g of sample. The sample undergoes calcination at 600°C for a period of 8 to 12 hours. After calcination the sample is quantitatively transferred to a 250ml beaker, 10ml of HCl 37% and 50ml of deionized water are added, and the ashes are digested for 20 minutes at 60°C Once the digestion time has ended, the sample is quantitatively transferred to a 100ml volumetric flask.
If calcium are going to be analyzed, 10ml of 3% lantanum solution are added to the flask prior to make it to the volume.
For the analysis of iron, no lantanum is needed, so the volume is made to the mark with deionized water.

Brief description of the spectrophotometric method for the analysis of iron, calcium.

The AA spectrophotometer is calibrated with hollow cathode lamp and with certified standards depending on the element to analyzed.

Intercomparison of Infant Formula Analyses
SIM.QM-P5

VITAMIN C (Reduced Ascorbic Acid) in Ready to Feed Milk Based Infant Formula. 2,6 Dichloroindophenol Titrimetric Method

Brief description of the sample preparation.

Principle

Ascorbic acid is estimated by titration with colored oxidation-reduction indicator, 2,6 dichloroindophenol . EDTA is added as chelating agent to remove Fe and Cu interferences.

Reagents.

- Precipitan slution.
- Ascorbic acid standart solution.
- Indophenol standart solution.

Preparation of sample Assay Solution

Pipet 25-30 ml composite and equal volumen of precipitant solution (a), into 125 ml beaker. Desingnate total volumen as V ml of composite liquoted as E ml. Filter though folded rapid qualitative paper, 18.5 cm (Whatman No. 541, or equivalent). Designate filtrate as assay solution.

Reference: JAOAC 68, 514 (1985).
CAS – 50-81-7 (ascorbic acid).

TO:
Katherine Sharples
NIST
100 Bureau Drive, MS 8392
Gaithersburg, MD 20899-8392
USA

Intercomparison of Infant Formula Analyses
SIM.QM-P5

CALCIUM

Brief description of sample preparation procedures (including sample size, digestion conditions, etc)

0.5 g sample: AOAC method 985.35 – Ashed at 525 deg C until white and C-free (3-5). Dissolve in nitric acid. Dilute

Brief description of spectrometric method (including instrumentation, wavelengths, etc)
ICP-AES
Ca 317,933 nm

Brief description of uncertainty analysis (including of uncertainty and its standard uncertainty, degrees of freedom, type A or B, value of k, source of data [e.g., historical, calibration certificate], etc.)

Source of uncertainty	Standard uncertainly	Source of data
Mass sample	0,00028	Calibration certificate
Glassware	0,049	Tolerance of glassware
Temperature	0,00032	- Maximun variation of temperatura 3 degrees - Water spation coefficient
Concentration of Ca given by the equipment	≈ 0,1	The equipment (ICP-AES)

Degrees of freedom: Milk powder: 3
SRM 1846 Infant Formula: 2

Type of uncertainty: All type A
Value of κ: 2

Intercomparison of Infant Formula Analyses
SIM.QM-P5

IRON

Brief description of sample preparation procedures (including sample size, digestion conditions, etc)

0.5 g sample: AOAC method 985.35 = Ashed at 525 deg C until white and C-free (3-5). Dissolve in nitric acid. Dilute

Brief description of spectrometric method (including instrumentation, wavelengths, etc)
ICP-AES
Fe 239,562 nm

Brief description of uncertainty analysis (including of uncertainty and its standard uncertainty, degrees of freedom, type A or B, value of k, source of data [e.g., historical, calibration certificate], etc.)

Source of uncertainty	Standard uncertainly	Source of data
Mass sample	0,00028	Calibration certificate
Glassware	0,049	Tolerance of glassware
Temperature	0,00032	- Maximun variation of temperatura 3 degres - Water spation coefficient
Concentration of Fe given by the equipment	≈ 0,003	The equipment (ICP-AES)

Degrees of freedom: Milk powder: 3
SRM 1846 Infant Formula: 2

Type of uncertainty: All type A
Value of κ: 2

2002-04-18

Intercomparison of Infant Formula Analyses
SIM.QM-P5

CALCIUM

Brief description of sample preparation procedures (including samples size, digestion conditions, etc.)

Sample size 5,0000 g
Ashing procedure : Heat on hot plate until smoking ceases. Place sample in 500 °C furnace for time necessary to obtain ash that is white and free from carbone (10 h) .
Dissolve the sample in 5 mL HCl conc, add solution to 100,00 ml vol. Flask.

Brief description of spectrometric method (including instrumentation, wavelengths, etc.)

Calcium in Infant Formula, method of standard additions :
Concentrations: **0** mg/ L, **1** mg/ L, **2** mg/ L and **3** mg/ L in Ca^{+2}

Used lanthanum cloride 0,1 % w / v, to inhibit chemicals interferences and KCl 0,1 % w / v to controlled the slight ionization occurs in the air-acetylene flame.

Instrumentation: Atomic Absorption Spectrometer, Perkin Elmer Instruments, A Analyst 300
λ = 422,7 nm, Flow C_2H_2 2,4 L/ min, Air 10 L/ min

Brief description of uncertainly analysis

Combined Type A	154	
Combined Type B	0,0041	
Combined Uncertainty (uc)	154	
Coverage Factor (k)	2	
Expanded Uncertainly U		308
Effective Degrees of Freedom		> 30

2002-04-18

Intercomparison of Infant Formula Analyses
SIM.QM-P5

IRON

Brief description of sample preparation procedures (including samples size, digestion conditions, etc.)

Sample size 5,0000 g
Ashing procedure : Heat on hot plate until smoking ceases. Place sample in 500 °C furnace for time necessary to obtain ash that is white and free from carbone (10 h) .
Dissolve the sample in 5 mL HCl conc, add solution to 100,00 ml vol. Flask.

Brief description of spectrometric method (including instrumentation, wavelengths, etc.)

Iron in Infant Formula, method of standard additions :
Concentrations: **0** mg/ L, **3** mg/ L, and **5** mg/ L in Fe^{+3}

Instrumentation: Atomic Absorption Spectrometer, Perkin Elmer Instruments, A Analyst 300
λ = 248,3 nm, Flow C_2H_2 2,2 L/ min, Air 10 L/ min

Brief description of uncertainly analysis

Combined Type A	4,4
Combined Type B	0,0015
Combined Uncertainty (uc)	4,4
Coverage Factor (k)	2
Expanded Uncertainly U	8,8

Effective Degrees of Freedom > 30

INTERCOMPARISON OF INFANT FORMULA ANALYSIS
SIM.QM-P5

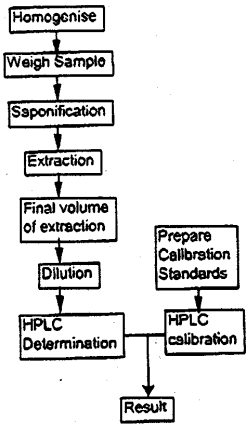
VITAMIN A
Brief description of sample preparation procedures (including sample size, internal standard, saponification conditions, extracting solvent, etc.

Measurement procedure

Sample size = 5,0000 g

Saponification conditions:
60 mL of Ethanol +
20 mL of KOH 50% p/v aqueous
Time: 30 minutes under reflux

Extraction:
Solvent: Ethyl ether
Evaporate to drynees in rotary evaporator under partial vacuum at a water-bath temperature of aproximately 50° C
Evaporate the last traces of water by repeated addition of ethanol
Dissolve the residue in n-hexane.
This solution is ready for injection into the HPLC



Brief description of chromatographic method (including analytical column, mobile phase, detector, wavelengths, etc.)

Analytical column: Supelcosil LC-8, 25 cm X 4,6 mm, 5 um
Mobile phase: n-hexane containing 2 percent isopropanol, isocratic
Flow rate: 0,75 mL/min
Temperature: 20° C
Injection volume: 20 uL

Brief description of uncertainty analysis (including source of uncertainty and its standard uncertainty, degrees of freedom, type A or B, value of k, source of data [e.g. historical, calibration certificate], etc.)

Measurand

r = [Co] [V] [Frep] [Fdil] [1.7] [0.2]/([m] [Rec])

Uncertainties in Vitamin A analysis, Milk Powder: Packet 1, Sample 1						
Description	Value x	Standard uncertainty u(x)	Relative uncertainty u(x)/x	Type	Degrees of freedom	Source of Data
Co: Content of Vitamin A in the extraction solution Packet 1, Sample 1	6.507	0.0390	0.00599	A	4	In house
V: Volume of the extraction solution	10.00	0.15	0.015	B		Certificate
m : mass of the sample	5.0068	0.003	0.001	B		Certificate
Rec: Bias	0.889	0.0611	0.062	A	4	In house
Frep: Repeatability	1	0.075	0.075	A	4	In house
Fdil: Dilution Factor (Not applicable to this sample)	10	0.063	0.006	B		Certificate

Uncertainties in Vitamin A analysis, Milk Powder: Packet 1, Sample 2						
Description	Value	Standard	Relative	Type	Degrees of	Source of

Co: Content of Vitamin A in the extraction solution Packet 1, Sample 2	5.136	Uncertainty 0.0301	Uncertainty 0.00586	A	freedom 4	Data In house
V: Volume of the extraction solution	10.00	0.15	0.015	B		Certificate
m : mass of the sample	5.0059	0.003	0.001	B		Certificate
Rec: Bias	0.989	0.0611	0.062	A	4	In house
Frep: Repeatability	1	0.075	0.075	A	4	In house
Fdil: Dilution Factor (Not applicable to this sample)	10	0.063	0.006	B		Certificate

Uncertainties in Vitamin A analysis, Milk Powder: Packet 2, Sample 1

Description	Value x	Standard uncertainty u(x)	Relative uncertainty u(x)/x	Type	Degrees of freedom	Source of Data
Co: Content of Vitamin A in the extraction solution Packet 1, Sample 2	0.603	0.0117	0.01940	A	4	In house
V: Volume of the extraction solution	10.00	0.15	0.015	B		Certificate
m : mass of the sample	5.0093	0.003	0.001	B		Certificate
Rec: Bias	0.989	0.0611	0.062	A	4	In house
Frep: Repeatability	1	0.075	0.075	A	4	In house
Fdil: Dilution Factor	10	0.063	0.006	B		Certificate

Uncertainties in Vitamin A analysis, Milk Powder: Packet 2, Sample 2

Description	Value x	Standard uncertainty	Relative uncertainty	Type	Degrees of freedom	Source of Data
Co: Content of Vitamin A in the extraction solution Packet 1, Sample 2	0.588	0.0117	0.01857	A	4	In house
V: Volume of the extraction solution	10.00	0.15	0.015	B		Certificate
m : mass of the sample	4.9998	0.003	0.001	B		Certificate
Rec: Bias	0.989	0.0611	0.062	A	4	In house
Frep: Repeatability	1	0.075	0.075	A	4	In house
Fdil: Dilution Factor	10	0.063	0.006	B		Certificate

Uncertainties in Vitamin A analysis, Milk Powder: Packet 3, Sample 1

Description	Value x	Standard uncertainty u(x)	Relative uncertainty u(x)/x	Type	Degrees of freedom	Source of Data
Co: Content of Vitamin A in the extraction solution Packet 1, Sample 2	0.507	0.0118	0.02327	A	4	In house
V: Volume of the extraction solution	10.00	0.15	0.015	B		Certificate
m : mass of the sample	0.989	0.0611	0.062	A	4	In house
Rec: Bias	1	0.075	0.075	A	4	In house
Frep: Repeatability	10	0.063	0.006	B		Certificate
Fdil: Dilution Factor						

Uncertainties in Vitamin A analysis, Milk Powder: Packet 3, Sample 2

Description	Value x	Standard uncertainty u(x)	Relative uncertainty u(x)/x	Type	Degrees of freedom	Source of Data
Co: Content of Vitamin A in the extraction solution Packet 1, Sample 2	0.431	0.0119	0.02781	A	4	In house
V: Volume of the extraction solution	10.00	0.15	0.015	B		Certificate
m : mass of the sample	5.0058	0.003	0.001	B		Certificate
Rec: Bias	0.989	0.0611	0.062	A	4	In house
Frep: Repeatability	1	0.075	0.075	A	4	In house
Fdil: Dilution Factor	10	0.063	0.006	B		Certificate

Uncertainties in Vitamin A analysis, SRM 1846 Infant Formular: Packet 1, Sample 1

Description	Value x	Standard uncertainty u(x)	Relative uncertainty u(x)/x	Type	Degrees of freedom	Source of Data
Co: Content of Vitamin A in the extraction solution	0.663	0.0117	0.01765	A	4	In house

Packet 1, Sample 2						
V: Volume of the extraction solution	10.00	0.15	0.075	B		Certificate
Rec: Bias	0.989	0.0611	0.062	A	4	In house
Frep: Repeatability	1	0.075	0.075	A	4	In house
Fdil: Dilution Factor	10	0.063	0.006	B		Certificate

Uncertainties in Vitamin A analysis, SRM 1846 Infant Formular: Packet 2, Sample 1						
Description	Value x	Standard uncertainty u(x)	Relative uncertainty u(x)/x	Type	Degrees of freedom	Source of Data
Co: Content of Vitamin A in the extraction solution Packet 1, Sample 2	0.691	0.0117	0.01693	A	4	In house
V: Volume of the extraction solution	10.00	0.15	0.015	B		Certificate
m : mass of the sample	5.0008	0.003	0.001	B		Certificate
Rec: Bias	0.989	0.0611	0.062	A	4	In house
Frep: Repeatability	1	0.075	0.075	A	4	In house
Fdil: Dilution Factor	10	0.063	0.006	B		Certificate

Uncertainties in Vitamin A analysis, SRM 1846 Infant Formular: Packet 3, Sample 1						
Description	Value x	Standard uncertainty u(x)	Relative uncertainty u(x)/x	Type	Degrees of freedom	Source of Data
Co: Content of Vitamin A in the extraction solution Packet 1, Sample 2	0.748	0.0116	0.01551	A	4	In house
V: Volume of the extraction solution	10.00	0.15	0.015	B		Certificate
m : mass of the sample	4.9899	0.003	0.001	B		Certificate
Rec: Bias	0.989	0.0611	0.062	A	4	In house
Frep: Repeatability	1	0.075	0.075	A	4	In house
Fdil: Dilution Factor	10	0.063	0.006	B		Certificate

RESULTS: MILK POWDER			
	Packet 1	Packet 2	Packet 3
Sample 1	6.624	7.544	5.184
Sample 2	5.226	6.056	4.383

Mean of six results: (5.61 ± 1.97) ug Retinol/g
The reported uncertainty is an expended uncertainty calculated using a coverage factor of 2
Confidence level = 95%

RESULTS: SRM 1846			
	Packet 1	Packet 2	Packet 3
Sample 1	6.753	7.036	7.618

Mean of six results: (7.14 ± 1.97) ug Retinol/g
The reported uncertainty is an expended uncertainty calculated using a coverage factor of 2
Confidence level = 95%

INTERCOMPARISON OF INFANT FORMULA ANALYSIS
SIM.QM-P5

CALCIO

Brief description of sample preparation procedures (including sample size, internal standard, saponification conditions, extracting solvent, etc.

La muestra fue homogenizada, previo a la pesada. Se pesó en balanza analítica aproximadamente un gramo, sobre un crisol de porcelana previamente tarado. Analizando un blanco fortificado y material de referencia fortificado como parámetros de control. El blanco fortificado y el material de referencia fortificado se colocaron en un hotplate calentando hasta sequedad. La bateria de análisis se colocó luego en mufla a 525° C por 5 horas, obteniéndose en esta etapa cenizas grises. Por lo que se procedió a la adición de 2 mL de ácido nítrico concentrado evaporando en hotplate hasta sequedad. Luego se colocaron nuevamente en la mufla a 525° C por dos horas más. Después de enfriados los crisoles se humedecieron las cenizas con una pequeña porción de agua seguida de la adición de 5 mL de ácido nitrico 1 N, transfiriéndose a un volumétrico de 25 mL, aforando con el mismo ácido, tomando un mililitro de esta solución para la determinación de calcio, adicionando en esta etapa 2 mL de cloruro de lantano.2.5 % llevando a un volumen final de 25 mL con agua destilada.

Brief description of chromatographic method (including analytical column, mobile phase, detector, wavelengths, etc.)

Para la cuantificación de calcio se utilizó un espectrofotómetro de absorción atómica Perkin Elmer modelo 3100, utilizando una longitud de onda de 422.7 nm, un slit de 0.7 y la respectiva curva de calibracion (0.0, 2.0, 4.0, 8.0 y 10.0 ppm)

Brief description of uncertainty analysis (including source of uncertainty and its standard uncertainty, degrees of freedom, type A or B, value of k, source of data [e.g. historical, calibration certificate], etc.)

Mensurando:

Conc Ca = x = C AA x V aforo M x V aforo Aliq /(g Muestra x Alicuota x Recup.)

Incertidumbres en Análisis de Calcio, Milk Powder: Packet 1, Sample 1						
Descripción	Valor x	Incerteza Estándar u(x)	Incerteza Relativ u(x)/x	Tipo	Grados de Libertad	Fuente de Datos
C AA: Concentración leida en Espectro-fotómetro	12.020	0.112	0.00931	A	3	En casa
V aforo M: Volumen aforo de muestra	25.00	0.05	0.002	B		Certificado
V aforo Aliq.: Volumen de aforo alicuota	25.00	0.05	0.002	B		Certificado
g Muestra: gramos de muestra	1.0049	0.0030	0.003	B		Certificado
Aliq: Alicuota a diluir	1.00	0.04	0.040	B		Certificado
Recup. Recuperación	1.059	0.037	0.035	A	7	En casa

Incertidumbres en Análisis de Calcio, Milk Powder: Packet 1, Sample 2						
Descripción	Valor x	Incerteza Estándar u(x)	Incerteza Relativ u(x)/x	Tipo	Grados de Libertad	Fuente de Datos
C AA: Concentración leida en Espectro-fotómetro	12.450	0.115	0.00921	A	4	En casa
V aforo M: Volumen aforo de muestra	25.00	0.05	0.002	B		Certificado
V aforo Aliq.: Volumen de aforo alicuota	25.00	0.05	0.002	B		Certificado
g Muestra: gramos de muestra	1.0458	0.0030	0.003	B		Certificado
Aliq: Alicuota a diluir	1.00	0.04	0.040	B		Certificado
Recup. Recuperación	1.059	0.037	0.035	A	7	En casa

Incertidumbres en Análisis de Calcio, Milk Powder: Packet 2, Sample 1						
Descripción	Valor x	Incerteza Estándar u(x)	Incerteza Relativ u(x)/x	Tipo	Grados de Libertad	Fuente de Datos
C AA: Concentración leida en Espectro-fotómetro	12.160	0.113	0.00928	A	4	En casa
V aforo M: Volumen aforo de muestra	25.00	0.05	0.002	B		Certificado
V aforo Aliq.: Volumen de aforo alicuota	25.00	0.05	0.002	B		Certificado
g Muestra: gramos de muestra	1.0151	0.0030	0.003	B		Certificado
Aliq: Alicuota a diluir	1.00	0.04	0.040	B		Certificado
Recup. Recuperación	1.059	0.037	0.035	A	7	En casa

Incertidumbres en Análisis de Calcio, Milk Powder: Packet 2, Sample 2						
Descripción	Valor x	Incerteza Estándar u(x)	Incerteza Relativ u(x)/x	Tipo	Grados de Libertad	Fuente de Datos
C AA: Concentración leida en Espectro-fotómetro	12.400	0.114	0.00922	A	4	En casa
V aforo M: Volumen aforo de muestra	25.00	0.05	0.002	B		Certificado
V aforo Aliq.: Volumen de aforo alicuota	25.00	0.05	0.002	B		Certificado

g Muestra: gramos de muestra	1.0362	0.0030	0.003	B		Certificado
Aliq: Alicuota a diluir	1.00	0.04	0.040	B		Certificado
Recup. Recuperación	1.059	0.037	0.035	A	7	En casa

Incertidumbres en Análisis de Calcio, Milk Powder: Packet 3, Sample 1						
Descripción	Valor x	Incerteza Estándar u(x)	Incerteza Relativ u(x)/x	Tipo	Grados de Libertad	Fuente de Datos
C AA: Concentración leida en Espectro-fotómetro	12.920	0.118	0.00911	A	4	En casa
V aforo M: Volumen aforo de muestra	25.00	0.05	0.002	B		Certificado
V aforo Aliq.: Volumen de aforo alicuota	25.00	0.05	0.002	B		Certificado
g Muestra: gramos de muestra	1.0929	0.0030	0.003	B		Certificado
Aliq: Alicuota a diluir	1.00	0.04	0.040	B		Certificado
Recup. Recuperación	1.059	0.037	0.035	A	7	En casa

Incertidumbres en Análisis de Calcio, Milk Powder: Packet 3, Sample 2						
Descripción	Valor x	Incerteza Estándar u(x)	Incerteza Relativ u(x)/x	Tipo	Grados de Libertad	Fuente de Datos
C AA: Concentración leida en Espectro-fotómetro	11.960	0.112	0.00933	A	4	En casa
V aforo M: Volumen aforo de muestra	25.00	0.05	0.002	B		Certificado
V aforo Aliq.: Volumen de aforo alicuota	25.00	0.05	0.002	B		Certificado
g Muestra: gramos de muestra	1.0050	0.0030	0.003	B		Certificado
Aliq: Alicuota a diluir	1.00	0.04	0.040	B		Certificado
Recup. Recuperación	1.059	0.037	0.035	A	7	En casa

Incertezas en Análisi de Calcio, SRM 1846 Infant Formular: Packet 1, Sample 1						
Descripción	Valor x	Incerteza Estándar u(x)	Incerteza Relativ u(x)/x	Tipo	Grados de Libertad	Fuente de Datos
C AA: Concentración leida en Espectro-fotómetro	6.150	0.087	0.01422	A	4	En casa
V aforo M: Volumen aforo de muestra	25.00	0.05	0.002	B		Certificado
V aforo Aliq.: Volumen de aforo alicuota	25.00	0.05	0.002	B		Certificado
g Muestra: gramos de muestra	1.0389	0.0030	0.003	B		Certificado
Aliq: Alicuota a diluir	1.00	0.04	0.040	B		Certificado
Recup. Recuperación	1.059	0.037	0.035	A	7	En casa

Incertezas en Análisi de Calcio, SRM 1846 Infant Formular: Packet 2, Sample 1						
Descripción	Valor x	Incerteza Estándar u(x)	Incerteza Relativ u(x)/x	Tipo	Grados de Libertad	Fuente de Datos
C AA: Concentración leida en Espectro-fotómetro	6.010	0.087	0.01451	A	4	En casa
V aforo M: Volumen aforo de muestra	25.00	0.05	0.002	B		Certificado
V aforo Aliq.: Volumen de aforo alicuota	25.00	0.05	0.002	B		Certificado
g Muestra: gramos de muestra	1.0141	0.0030	0.003	B		Certificado
Aliq: Alicuota a diluir	1.00	0.04	0.040	B		Certificado
Recup. Recuperación	1.059	0.037	0.035	A	7	En casa

Incertezas en Análisi de Calcio, SRM 1846 Infant Formular: Packet 3, Sample 1						
Descripción	Valor x	Incerteza Estándar u(x)	Incerteza Relativ u(x)/x	Tipo	Grados de Libertad	Fuente de Datos
C AA: Concentración leida en Espectro-fotómetro	6.250	0.088	0.01401	A	4	En casa
V aforo M: Volumen aforo de muestra	25.00	0.05	0.002	B		Certificado
V aforo Aliq.: Volumen de aforo alicuota	25.00	0.05	0.002	B		Certificado
g Muestra: gramos de muestra	1.0360	0.0030	0.003	B		Certificado
Aliq: Alicuota a diluir	1.00	0.04	0.040	B		Certificado
Recup. Recuperación	1.059	0.037	0.035	A	7	En casa

RESULTADOS: MILK POWDER

	Packet 1	Packet 2	Packet 3
Sample 1	7060	7070	6977
Sample 2	6956	7049	7024

Promedio de seis resultados: (7023 ± 583) ug Ca/g
La incertidumbre reportada es una incertidumbre expandida, con un factor de cobertura de 2,
lo cual da un nivel de confianza de aproximadamente 95%

RESULTADOS: SRM 1846

	Packet 1	Packet 2	Packet 3
Sample 1	3494	3498	3661

Promedio de tres resultados: (3518 ± 303) ug Ca/g
La incertidumbre reportada es una incertidumbre expandida, con un factor de cobertura de 2,
lo cual da un nivel de confianza de aproximadamente 95%

INTERCOMPARISON OF INFANT FORMULA ANALYSIS
SIM.QM-P5

HIERRO

Brief description of sample preparation procedures (including sample size, internal standard, saponification conditions, extracting solvent, etc.

La muestra fue homogenizada, previo a la pesada. Se pesó en balanza analítica aproximadamente un gramo, sobre un crisol de porcelana previamente tarado. Analizando un blanco fortificado y material de referencia fortificado como parámetros de control. El blanco fortificado y el material de referencia fortificado se colocaron en un hotplate calentando hasta sequedad. La batería de análisis se colocó luego en mufla a 525° C por 5 horas, obteniéndose en esta etapa cenizas grises. Por lo que se procedió a la adición de 2 mL de ácido nítrico concentrado evaporando en hotplate hasta sequedad. Luego se colocaron nuevamente en la mufla a 525° C por dos horas más. Después de enfriados los crisoles se humedecieron las cenizas con una pequeña porción de agua seguida de la adición de 5 mL de ácido nítrico 1 N, transfiriéndose a un volumétrico de 25 mL, aforando con el mismo ácido.

Brief description of chromatographic method (including analytical column, mobile phase, detector, wavelengths, etc.)

Para la cuantificación de calcio se utilizó un espectrofotómetro de absorción atómica Perkin Elmer modelo 3100, utilizando una longitud de onda de 248.4 nm, un slit de 0.2 y la respectiva curva de calibracion (0.0, 1.0, 1.5, 2.0, 3.0, 4.0 y 6.0 ppm)

Brief description of uncertainty analysis (including source of uncertainty and its standard uncertainty, degrees of freedom, type A or B, value of k, source of data [e.g. historical, calibration certificate], etc.)

Mensurando:
Conc Fe = x = C AA x V aforo M /(g Muestra x Recup.) x Fvar

Incertidumbres en Análisis de Hierro, Milk Powder: Packet 1, Sample 1						
Descripción	Valor x	Incerteza Estándar u(x)	Incerteza Relativ u(x)/x	Tipo	Grados de Libertad	Fuente de Datos
C AA: Concentración leída en Espectro-fotómetro	4.290	0.0004306	0.00011	A	4	En casa
V aforo M: Volumen aforo de muestra	25.00	0.05	0.002	B		Certificado
g Muestra: gramos de muestra	1.0049	0.0030	0.003	B		Certificado
FVar: Variabilidad entre corridas	1.0000	1.8400	1.840	A	5	En casa
Recup. Recuperación	0.9810	0.0637	0.065	A	7	En casa

Incertidumbres en Análisis de Hierro, Milk Powder: Packet 1, Sample 2						
Descripción	Valor x	Incerteza Estándar u(x)	Incerteza Relativ u(x)/x	Tipo	Grados de Libertad	Fuente de Datos
C AA: Concentración leída en Espectro-fotómetro	4.470	0.0004849	0.00011	A	4	En casa
V aforo M: Volumen aforo de muestra	25.00	0.05	0.002	B		Certificado
g Muestra: gramos de muestra	1.0456	0.0030	0.003	B		Certificado
FVar: Variabilidad entre corridas	1.0000	1.8400	1.840	A	5	En casa
Recup. Recuperación	0.9810	0.06367	0.065	A	7	En casa

Incertidumbres en Análisis de Hierro, Milk Powder: Packet 2, Sample 1						
Descripción	Valor x	Incerteza Estándar u(x)	Incerteza Relativ u(x)/x	Tipo	Grados de Libertad	Fuente de Datos
C AA: Concentración leída en Espectro-fotómetro	4.370	0.0004831	0.00011	A	4	En casa
V aforo M: Volumen aforo de muestra	25.00	0.05	0.002	B		Certificado
g Muestra: gramos de muestra	1.0151	0.0030	0.003	B		Certificado
FVar: Variabilidad entre corridas	1.0000	1.8400	1.840	A	5	En casa
Recup. Recuperación	0.9810	0.06367	0.065	A	7	En casa

Incertidumbres en Análisis de Hierro, Milk Powder: Packet 2, Sample 2						
Descripción	Valor x	Incerteza Estándar u(x)	Incerteza Relativ u(x)/x	Tipo	Grados de Libertad	Fuente de Datos
C AA: Concentración leída en Espectro-fotómetro	4.470	0.000	0.00011	A	4	En casa
V aforo M: Volumen aforo de muestra	25.00	0.05	0.002	B		Certificado
g Muestra: gramos de muestra	1.0382	0.0030	0.003	B		Certificado
FVar: Variabilidad entre corridas	1.0000	1.8400	1.840	A	5	En casa
Recup. Recuperación	0.9810	0.06367	0.065	A	7	En casa

Incertidumbres en Análisis de Hierro, Milk Powder: Packet 3, Sample 1						
Descripción	Valor x	Incerteza Estándar u(x)	Incerteza Relativ u(x)/x	Tipo	Grados de Libertad	Fuente de Datos
C AA: Concentración leida en Espectro-fotómetro	4.530	0.0004861	0.00011	A	4	En casa
V aforo M: Volumen aforo de muestra	25.00	0.05	0.002	B		Certificado
g Muestra: gramos de muestra	1.0929	0.0030	0.003	B		Certificado
FVar: Variabilidad entre corridas	1.0000	1.8400	1.840	A	5	En casa
Recup. Recuperación	0.9810	0.06367	0.065	A	7	En casa

Incertidumbres en Análisis de Hierro, Milk Powder: Packet 3, Sample 2						
Descripción	Valor x	Incerteza Estándar u(x)	Incerteza Relativ u(x)/x	Tipo	Grados de Libertad	Fuente de Datos
C AA: Concentración leida en Espectro-fotómetro	4.390	0.0004835	0.00011	A	4	En casa
V aforo M: Volumen aforo de muestra	25.00	0.05	0.002	B		Certificado
g Muestra: gramos de muestra	1.0050	0.0030	0.003	B		Certificado
FVar: Variabilidad entre corridas	1.0000	1.8400	1.840	A	5	En casa
Recup. Recuperación	0.9810	0.06367	0.065	A	7	En casa

Incertezas en Análisi de Hierro, SRM 1846 Infant Formular: Packet 1, Sample 1						
Descripción	Valor x	Incerteza Estándar u(x)	Incerteza Relativ u(x)/x	Tipo	Grados de Libertad	Fuente de Datos
C AA: Concentración leida en Espectro-fotómetro	2.740	0.0004616	0.00017	A	4	En casa
V aforo M: Volumen aforo de muestra	25.00	0.05	0.002	B		Certificado
g Muestra: gramos de muestra	1.0389	0.0030	0.003	B		Certificado
FVar: Variabilidad entre corridas	1.0000	1.8400	1.840	A	5	En casa
Recup. Recuperación	0.9810	0.06367	0.065	A	7	En casa

Incertezas en Análisi de Hierro, SRM 1846 Infant Formular: Packet 2, Sample 1						
Descripción	Valor x	Incerteza Estándar u(x)	Incerteza Relativ u(x)/x	Tipo	Grados de Libertad	Fuente de Datos
C AA: Concentración leida en Espectro-fotómetro	2.520	0.0004599	0.00018	A	4	En casa
V aforo M: Volumen aforo de muestra	25.00	0.05	0.002	B		Certificado
g Muestra: gramos de muestra	1.0141	0.0030	0.003	B		Certificado
FVar: Variabilidad entre corridas	1.0000	1.8400	1.840	A	5	En casa
Recup. Recuperación	1.059	0.037	0.035	A	7	En casa

Incertezas en Análisi de Hierro, SRM 1846 Infant Formular: Packet 3, Sample 1						
Descripción	Valor x	Incerteza Estándar u(x)	Incerteza Relativ u(x)/x	Tipo	Grados de Libertad	Fuente de Datos
C AA: Concentración leida en Espectro-fotómetro	2.620	0.0004606	0.00018	A	4	En casa
V aforo M: Volumen aforo de muestra	25.00	0.05	0.002	B		Certificado
g Muestra: gramos de muestra	1.0360	0.0030	0.008	B		Certificado
FVar: Variabilidad entre corridas	1.0000	1.8400	1.840	A	5	En casa
Recup. Recuperación	1.059	0.037	0.035	A	7	En casa

RESULTADOS: MILK POWDER

	Packet 1	Packet 2	Packet 3
Sample 1	107.3	109.7	105.6
Sample 2	108.9	109.7	111.3

Promedio de seis resultados: (108.8 ± 4.0) ug Fe/g
La incertidumbre reportada es una incertidumbre expandida, con un factor de cobertura de 2,
lo cual da un nivel de confianza de aproximadamente 95%

RESULTADOS: SRM 1846

	Packet 1	Packet 2	Packet 3
Sample 1	67.2	63.3	64.4

Promedio de tres resultados: (65.0 ±4.0) ug Fe/g
La incertidumbre reportada es una incertidumbre expandida, con un factor de cobertura de 2,
lo cual da un nivel de confianza de aproximadamente 95%

CALCIUM

Brief description of sample preparation procedures (including sample size, digestion conditions, etc.)

The sample was mixed. 1 g of the sample was accurately weighted (in duplicate) into porcelain dishes. The sample was dried for two hours at 105°C. The dishes were transferred to a muffle furnace and ignited for two hours at 550°C. The ash was treated with 5 ml of hydrochloric acid (1 +1) and evaporated to dryness. This step was repeated twice. It was transferred into a 100 ml volumetric flask and diluted to mark with water.
1 ml was taken and transferred into a 25 ml volumetric flask and 2.5 ml KCl 2.5% m/v were added and additional water was added until the mark was reached.

Brief description of spectrometric method (including instrumentation, wavelengths, etc.)

The standard curve was made as follows: 1,2,3,4 and 5 mg/L.
The reading was made in an atomic absorption spectrophotometer Perkin Elmer AA300.
The operating conditions were:

Lamp Current (mA):	30
Wavelength (mm)	422.7
Slit width (mm)	0.7
Acetylene (L/min)	3.0
Air (L/min)	10.0

Brief description of uncertainty analysis (including source of uncertainty and its standard uncertainty, degrees of freedom, type A or B, value of k, source of data [e.g., historical, calibration certificate], etc.)

The uncertainty data reported are only the standard deviation computed from the results obtained for the six samples resulting from the three packets.

Intercomparison of Infant Formula Analyses
SIM.QM-P5

IRON

Brief description of sample preparation procedures (including sample size, digestion conditions, etc.)

The sample was mixed. 1 g of the sample was accurately weighted (in duplicate) into porcelain dishes. The sample was dried for two hours at 105°C. The dishes were transferred to a muffle furnace and ignited for two hours at 550°C. The ash was treated with 5 ml of hydrochloric acid (1 +1) and evaporated to dryness. This step was repeated twice. It was transferred into a 100 ml volumetric flask and diluted to mark with water.

Brief description of spectrometric method (including instrumentation, wavelengths, etc.)
The standard curve was made as follows: 1,2,3,4,5 and 6 mg/L.
The reading was made in an atomic absorption spectrophotometer Perkin Elmer AA300.
The operating conditions were:

Lamp Current (mA):	30
Wavelength (mm)	448.3
Slit width (mm)	0.2
Acetylene (L/min)	3.0
Air (L/min)	10.0

Brief description of uncertainty analysis (including source of uncertainty and its standard uncertainty, degrees of freedom, type A or B, value of k, source of data [e.g., historical, calibration certificate], etc.)

The uncertainty data reported are only the standard deviation computed from the results obtained for the six samples resulting from the three packets.

LABORATORIO N° 3, EL SALVADOR

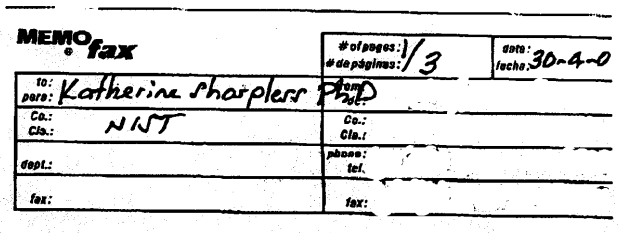
ANÁLISIS DE INTERCOMPARACIÓN DE FÓRMULA INFANTIL SIM QM – P5

ANÁLISIS DE HIERRO

MÉTODO ESPECTROFOTOMÉTRICO AOAC 944.02, 16TH EDICIÓN

PREPARACIÓN DE LA MUESTRA.

Se coloca la muestra en un beaker de 1000mL y se mezcla. Pesar 4.0g de muestra SIM 8 P5 – 2 y colocarlos en un crisol de porcelana de 50mL de capacidad. Incinerar la muestra en una muffla a 550° C, enfriar en un desecador. Disolver el residuo con 5mL de ácido clorhídrico y calentar hasta sequedad. Agregar 2mL de ácido clorhídrico y digerir por 5 minutos. Trasladar el líquido del crisol cuantitativamente a un balón de 100.0mL, diluir con agua a 100mL y homogenizar.


MEMO fax

to: Katherine Sharpless PhD	# of pages: 3	date: 30-4-0
Co.: NIST	Co.:	
dept.:	phone:	
fax:	fax:	

MÉTODO ESPECTROFOTOMÉTRICO

Reacción colorimétrica con ortofenantrolina.

REACTIVOS.

- Solución de hidroxilamina al 10%
- Buffer acetato de sodio solución
- Solución de ortofenantrolina

PROCEDIMIENTO

Trasladar 5.0mL de la dilución de la muestra a un balón de 25.0mL y agregar 1.0mL de hidroxilamina, dejar reposar durante 5 minutos, agregar 5.0mL de buffer acetato de sodio solución y 1.0mL de ortofenantrolina, aforar con agua y homogenizar. Leer en el espectrofotómetro en una celda de 1cm, a la longitud de onda máxima de 510nm, contra un blanco de reactivos.

Determinar la concentración de hierro comparado con un estándar de hierro de 2µg/mL.

MEMO fax

to: para Katherine Shasplers	# of pages: 2/3 / # de páginas:
Co.: Cía.:	from: Pilar D.
dept.:	phone: tel:
fax:	fax:

CÁLCULO.

$$CFe\mu g/g = \frac{Abm \times Cst \times Fd}{Abst \times Pm\ (g)}$$

Donde:

Abm = Absorbancia de la muestra

Abst = Absorbancia del estándar

Cst = Concentración del estándar

Pm (g) = Peso de la muestra en gramos

Fd = Factor de dilución de la muestra

DESCRIPCIÓN DEL ANÁLISIS DE LA INCERTIDUMBRE.

La incertidumbre expandida se calcula de acuerdo al método de la guía EURACHEM / CITAC con un nivel de confianza del 95%.

Las fuentes principales de incertidumbre se sacaron de la fórmula utilizada para la cuantificación.

Fuentes de Incertidumbre	Incertidumbre estándar relativa	Tipo de evaluación
Concentración del estándar	0.00182	B
Absorbancia de la muestra	0.012	B
Absorbancia del estándar	0.012	B
Peso de la muestra	0.000055	B

Valor de K = 2

FUENTES DE DATOS.

Certificados de calibración.

Intercomparison of Infant Formula Analyses
SIM.QM-P5

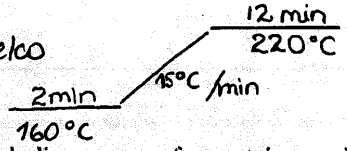
LINOLEIC ACID AND LINOLENIC ACID

Brief description of sample preparation procedures (including sample size, internal standard, hydrolysis conditions, etc.)

- AOAC Official Method 905.02 FAT IN MILK Roese-Gotlieb Method (16the
  Fat extraction: 10 mL of ready to feed milk (128,5g/L) in an extraction flask + 1.25 mL NH_4OH + 10 mL alcohol + 25mL ether + 25 mL petroleum ether (2x). Evaporate solvents and dry fat (oven 105°C)
- FAMEs EXTRACTION: 4 drops of fat + 0.8 mL hexane + 0.5 mL 2M KOH in Methanol, vortex 8 minutes. let stand 10 min. Pipet 0.3mL hexane phase and dilute with 4mL hexane add 1 g Na_2SO_4, mix and let stand 10min; Transfer to vial and inject.

Brief description of chromatographic method (including analytical column, detector, etc.)

- Equipment: Agilent GC 6890
- Column: No. J&W SC DB-23 30m x 250µm x 0.25µm
- Detector: FID 300°C H_2 flow: 30mL/min; AIR flow: 400 mL/min; N_2 Makeup
- Inlet: split mode 250°C; 16.43 psi, total flow 53.4 mL/min
- Flow: constant flow: 1.0 mL/min
- Carrier gas: N_2
- Standard: RM6 FAME Supelco LA95912
- Oven temperatures:



Brief description of uncertainty analysis (including source of uncertainty and its standard uncertainty, degrees of freedom, type A or B, value of k, source of data [e.g., historical, calibration certificate], etc.)

Calculation of standard deviation

** Values calculated for SRM 1846 were calculated based on the data of fat content of the reference sample (27.1 % fat).

$$\begin{matrix} C18:2 \\ C18:3 \end{matrix} = \begin{matrix} \% \text{ (FAME)} \\ \text{from the} \\ \text{Chromatogram} \end{matrix} \times \frac{27}{100}$$

Intercomparison of Infant Formula Analyses
SIM.QM-P5

IRON

Brief description of sample preparation procedures (including sample size, digestion conditions, etc.)

Sample weight (powder milk) aprox. 10g dry ashing (Furnace 580°C. Disolve ash in HCl /HNO_3 (2:1) heat at low temperature to evaporate solvents (do not bring to dryness). Ash solution is done with Mehlich (1.4mL H_2SO_4 + 8.33mL HCl for 2L.) for Fe.

Brief description of spectrometric method (including instrumentation, wavelengths, etc.)

Instrument: AA Spectra 5 Varian
Wavelengths: 248.3 nm
Sensitivity: 0.06 ppm Fe

Brief description of uncertainty analysis (including source of uncertainty and its standard uncertainty, degrees of freedom, type A or B, value of k, source of data [e.g., historical, calibration certificate], etc.)

Calculation of standard deviation

VITAMIN A

Brief description of sample preparation procedures (including sample size, internal standard, saponification conditions, extracting solvent, etc.)

AOAC Official Method 992.04 Vitamin A in Milk LIQUID CHROMATOGRAPHIC METHOD

40 mL ready to feed sample (128.5g/L) is digested with KOH solution (10% in ethanol) and pyrogallol solution (2% in ethanol) during 18h. in flask (covered with aluminum foil). Pipet 3mL of digested sample and extract Vitamin A with huxane (3x). Evaporate solvent under N_2 and dilute the residue with 1mL of heptano. Transfer to vial and inject.

Brief description of chromatographic method (including analytical column, mobile phase, detector, wavelengths, etc.)

Equipment: PerkinElmer Serie 200 Lc Pump. Detector 785A UV/VIS
Column: 5µm Silica 15cm.
Mobile phase: 95% Heptane 5% Isopropanol HPLC grades
Detector wavelengths: 340nm
flow: 1mL/min
Loop: 200µL
Injection volume: 400µL
Standard: All trans retinol SIGMA R-7632 (HPLC)

Brief description of uncertainty analysis (including source of uncertainty and its standard uncertainty, degrees of freedom, type A or B, value of k, source of data [e.g., historical, calibration certificate], etc.)

Calculation of standard deviation

CALCIUM

Brief description of sample preparation procedures (including sample size, digestion conditions, etc.)

Same procedure as for Iron. Solutions are made with La_2O_3 (1% in 5% HCl)

Brief description of spectrometric method (including instrumentation, wavelengths, etc.)

Instrument : AA Spectra 5 Varian
Wavelengths : 422.7 nm
Sensitivity : 0.01 ppm Ca

Brief description of uncertainty analysis (including source of uncertainty and its standard uncertainty, degrees of freedom, type A or B, value of k, source of data [e.g., historical, calibration certificate], etc.)

Calculation of standard deviation

Intercomparison of Infant Formula Analyses
SIM.QM-P5

CALCIUM

Brief description of sample preparation procedures (including sample size, digestion conditions, etc.)

- We weighted 2.72g of sample.
- Dissolved the sample in 210ml of deionizated water and 10ml of HCl in a 100ml volumetric flask.
- Heated for 1 hour.
- Diluted with water to volume.
- Pipeted 10ml from the above flask in a 100ml volumetric fask.
- Added Sr(N03)2 and diluted with water to volume.

Brief description of sprectrometric method (including instrumentation, wavelengths, etc.)

We did a standard calibration curve of 4 points: 1,2, 2.5 and 3mg/ml of calcium took readings at 422.7nm using an Atomic Absorption Spectrophotometer Shimadzu AA-6701 controlled by a flow fitter and an automatic burner positioning software. Each reading was done three times.

Brief description of uncertainty analysis (including source of uncertainty and its standard uncertainty, degrees of freedom, type A or B, value of k, source of data [e.g., historical, calibration certificate], etc.)

We took in consideration:

1. Glassware (toerance/$\sqrt{3}$)
2. The uncertainty of the lineality of the balance ($0.22/\sqrt{3}$) where 0.22 is the value that it is within the range $\pm$ 0.03mg.

We use the following formula to calculate uncertainty (for repetitions less than 10)

$$u(x) = \frac{s(xk)}{\sqrt{n}} \; t$$

In which uses a Standard Deviation of the media, the number of repetitions and a value of t-student, k=2, and a level of confiance of 95.45%.

Intercomparison of Infant Formula Analyses
SIM.QM-P5

IRON

Brief description of sample preparation procedures (including sample size, digestion conditions, etc.)

- Weighted 3.0g of the sample.
- Dissolved the sample in 20ml of deionizated water and 10ml of HCI in a 100ml volumetric flask.
- Heated for 1 hour.
- Added 1ml of H3P04
- Diluted with water to volume.

Brief description of sprectrometric method (including instrumentation, wavelengths, etc.)

We did a standard calibration curve of 5 points: 2, 3, 4, 5, 8 ug/ml of iron took readings at 248.3nm using an Atomic Absorption Spectophotometer Shimadzu AA-6701 controlled by a flow fitter and an automatic burner positioning software each reading was done three times.

Brief description of uncertainty analysis (including source of uncertainty and its standard uncertainty, degrees of freedom, type A or B, value of k, source of data [e.g., historical, calibration certificate], etc.)

We took in consideration:

1. Glassware (toerance/$\sqrt{3}$)
2. The uncertainty of the lineality of the balance ($0.22/\sqrt{3}$) where 0.22 is the value that it is within the range $\pm$ 0.03mg.

We use the following formula to calculate uncertainty (for repetitions less than 10)

$$u(x) = \frac{s\,(xk)}{\sqrt{n}}\ t$$

In which uses a Standard Deviation of the media, the number of repetitions and a value of t-student, k=2, and a level of confiance of 95.45%.

Intercomparison of Infant Formula Analyses
SIM.QM-P5

VITAMINA C

Brief description of sample preparation procedures (including sample size, digestion conditions, etc.)

Sample Weighted 1.75g of sample, added 50ml of mobile phase (see below) shook for two hours by mechanical means and diluted to volume with mobile phase to a 100ml volumetric flask filter.

Standard Weighted 20mg of Ascorbic Acid in a 100ml volumetric flask and diluted it with mobile phase to volume pipeted 10ml in a 100ml volumetric flask and diluted to volume with mobile phase.

Brief description of sprectrometric method (including instrumentation, wavelengths, etc.)

We used a HP 1100 series Liquid Chromatograph equipped with: quarternary pump vacuum degasser, automatic injector, autosampler, thermostatted column compartment, diode array detector, HPChem Station for LC systems.

Column: HP0DS Hypersil, LC systems
Flow: 1.5ml/min
Wavelength: 244nm
Iny. Vol.: 50ml

Brief description of uncertainty analysis (including source of uncertainty and its standard uncertainty, degrees of freedom, type A or B, value of k, source of data [e.g., historical, calibration certificate], etc.)

We took in consideration:

1. Glassware (toerance/$\sqrt{3}$)
2. The uncertainty of the lineality of the balance ($0.22/\sqrt{3}$) where 0.22 is the value that it is within the range $\pm$ 0.03mg.

We use the following formula to calculate uncertainty (for repetitions less than 10)

$$u(x) = \frac{s\,(xk)}{\sqrt{n}}\ t$$

In which uses a Standard Deviation of the media, the number of repetitions and a value of t-student, k=2, and a level of confiance of 95.45%.

Intercomparison of Infant Formula Analyses
SIM.QM-P5

VITAMIN C

Brief description of sample preparation procedures (including sample size, internal standard, saponification conditions, extracting solvent, etc.)

Approximately 5.0000g of well mixed sample was weighed and transferred to 100mL volumetric flask.This was made up to the mark with precipitant solution* then shaken vigourously for about 2 mins then filtered. 10mL of filtrate was pippetted and titrated with Indophenol Standard Solution(standardised using Ascorbic acid standard solution, 1mg/mL).

* Made using equal volumes of :
  1. 0.9g EDTA in 250mL and
  2. 15g glacial metaphosphoric acid + 40mL glacial acetic acid in 250mL.

Brief description of chromatographic method (including analytical column, mobile phase, detector, wavelengths, etc.)

The method used was not a chromatographic method.

The method used was the 2,6-dichloroindophenol Titrimetric Method where Vitamin C (Reduced Ascorbic Acid) is estimated by titration with coloured oxidation-reduction indicator, 2,6-dichloroindophenol. EDTA is added as chelating agent to remove Fe and Cu interferences.

Reference: 1. Methods of analysis for nutritional labelling(1993)
2. JAOAC 68, 514(1985)

Brief description of uncertainty analysis (including source of uncertainty and its standard uncertainty, degrees of freedom, type A or B, value of k, source of data [e.g., historical, calibration certificate], etc.)

Source and Standard uncertainty :
Balance ±1% of weight used
100mL volumetric flask ±0.08
10mL pipette ±0.04
Burette ±0.05

Type B Evaluation of Standard Uncertainty was used

Value of k = 2 (95% level of confidence)

Intercomparison of Infant Formula Analyses
SIM.QM-P5

CALCIUM AND IRON

Brief description of sample preparation procedures (including sample size, digestion conditions, etc.)

Approximately 2.0000g of well mixed sample was weighed in a ceramic crucible.This was ashed at 600°C overnight.
The ash was digested using hydrochloric acid (2+10),cooled and filtered into a 100mL volumetric flask containing 10mL lanthanum chloride solution (1%) and then made up to mark using deionised water.

A dilution of 1 in 50 was made for analysis of calcium.

Brief description of spectrometric method (including instrumentation, wavelengths, etc.)

Atomic Absorption (Thermo Jarrell Ash Smith-Hieftje 11)
Air - Acetylene flame
Smith-Hieftje background was used.

Wavelength: Calcium - 422.7
Iron - 248.3

Brief description of uncertainty analysis (including source of uncertainty and its standard uncertainty, degrees of freedom, type A or B, value of k, source of data [e.g., historical, calibration certificate], etc.)

Source of uncertainty & Standard Uncertainty :
Balance ±1% of weight used
100mL volumetric flask ±0.08
2mL pipette(used for Calcium dilution) ±0.006

%RSD obtained from Atomic Absorption Spectrophotometer for each reading.

Type B Evaluation of Standard Uncertainty was used

Value of k = 2 (95% level of confidence)

Intercomparison of Infant Formula Analyses
SIM.QM-P5

CALCIUM

Brief description of sample preparation procedures (including sample size, digestion conditions, etc.)

1 g of the sample was ashed at 600°C for 14 hours in a muffle furnace. The resulting carbon free ash was digested on a hot plate with 1:5 HCl until boiling had occurred. This was then filtered through Whatman #541 filter paper into a 100ml volumetric flask and made up to volume. 10ml of a 5% solution of Lanthanum Chloride was added to a dilution of the above solution and made up to volume. This solution was used to determine the concentration of Calcium in the sample.

Brief description of spectrometric method (including instrumentation, wavelength, etc.)

SOLAAR Atomic Absorption Spectrophotometer

Calcium wavelength: 422.7 nm

Brief description of uncertainty analysis (including source of uncertainty and its standard uncertainty, degrees of freedom, type A or B, valve of k, source of data [e.g., historical, calibration certificate}, etc.)

Sources of uncertainty

1. Weight of the sample (1% of the weight)
2. Volumes delivered by pipettes and volumetric flasks
   - 100ml volumetric flask ± 0.16
   - 50 ml volumetric flask ± .05
   - 2ml pipette ± 0.006
3. The RSD value for each analyte from the Spectrophotomer

The uncertainties are type A
Value of k = 2
Expanded Uncertainty, $U_T = k\ u_c$

CALCIUM RESULTS

SAMPLE CODE	VALUE mg/kg	STD UNCERTAINTY %RSD	A.A STD UNCERTAINTY %RSD	COMBINED UNCERTAINTY %RSD	EXPANDED UNCERTAINTY %RSD
Packet 1	6809	0.17	0.6	0.77	1.54
Packet 1	6523	0.17	0.6	0.77	1.54
Packet 2	5427	0.17	0.5	0.67	1.34
Packet 2	5428	0.17	0.4	0.57	1.14
Packet 3	5459	0.17	1.2	1.37	2.74
Packet 3	5500	0.17	1.1	1.27	2.54
SRM	3299	0.17	1.4	1.57	3.14
SRM	3052	0.17	1.2	1.37	2.74
SRM	3533	0.17	1.8	1.97	3.94

Intercomparison of Infant Formula Analyses
SIM.QM-P5

IRON

Brief description of sample preparation procedures (including sample size, digestion conditions, etc.)

1 g of the sample was ashed at 600°C for 14 hours in a muffle furnace. The resulting carbon free ash was digested on a hot plate with 1:5 HCl until boiling had occurred. This was then filtered through Whatman #541 filter paper into a 100ml volumetric flask and made up to volume. The solution was used to determine the concentration of Iron in the sample.

Brief description of spectrometric method (including instrumentation, wavelength, etc.)

SOLAAR Atomic Absorption Spectrophotometer

Iron wavelength: 248.3 nm

Brief description of uncertainty analysis (including source of uncertainty and its standard uncertainty, degrees of freedom, type A or B, valve of k, source of data {e.g., historical, calibration certificate}, etc.)

Sources of uncertainty

1. Weight of the sample (1% of the weight)
2. Volumes delivered by volumetric flasks
   - 100ml volumetric flask ± 0.16
3. The RSD value for each analyte from the Spectrophotomer

The uncertainties are type A
Value of k = 2

Expanded Uncertainty, $U_T = k\ u_c =$

IRON RESULTS

SAMPLE CODE	VALUE mg/kg	STANDARD UNCERTAINTY %RSD	A.A STD UNCERTAINTY %RSD	COMBINED UNCERTAINTY %RSD	EXPANDED UNCERTAINTY %RSD
Packet 1	103	0.16	1.7	1.86	3.72
Packet 1	107	0.16	1.9	2.06	4.12
Packet 2	96	0.16	0.2	0.36	0.72
Packet 2	109	0.16	0.7	0.86	1.72
Packet 3	94	0.16	1.9	2.06	4.12
Packet 3	105	0.16	1.5	1.66	3.32
SRM	67.6	0.16	1.4	1.56	3.12
SRM	69.2	0.16	2.4	2.56	5.12
SRM	67.2	0.16	1.9	2.06	4.12

Intercomparison of Infant Formula Analyses
SIM.QM-P5

LINOLEIC AND LINOLENIC ACID

Brief description of sample preparation procedures (including sample size, internal standard, hydrolysis conditions, etc).

4 g of sample were weighted to extract the oil with the Accelerated Solvent Extraction (ASE) at the follow conditions:
Temp. 80°C, Cell Heat Up= 5 min,
Solvents: Hexane, Dichloromethane and Methanol (65, 25 and 10% respectively).

30 – 35 mg of sample oil were weighted into a culture tube containing the Internal Standard (methyl nonadecanoato C 19:0), the mix was saponified with 1.5 mL of methanolic Sodium hydroxide (NaOH) 0.5 N.
The esterification was made during 30 min at 100°C by adding BF3/ methanol reagent to prepare the methyl esters. Hexane containing 50 ppm of BHT antioxidant was added to extract the FAMES.

During both, the saponification and esterification process, the tubes were blanked with nitrogen.

Brief description of chromatographic method (including analytical column, detector, etc)

The analysis was made with a Carbowax-20M column with a flame-ionization detector (FID), the conditions of the method were:

On column injection port with 0.5 µL of injection volume, Detector 230 °C.
Oven temperature profile:
Initial temperature, 210°C
Initial hold time, 0 min
Program rate, 1°C/min
Final temperature, 228°C
Final hold time, 10 min
Carrier gas- chromatographic helium.

Brief description of uncertainty analysis (including source of uncertainty, degrees of freedom, type A or B, value of k, source of data [e.g., historical, calibration certificate], etc.)

The uncertainty is expressed as an expanded uncertainty, U at the 95 % level of confidence and is calculated as $U = k\, u_c$.*

Parameter	**Type**
Precision method	A

*(International Organization for Standarization (ISO), Guide to the expression of uncertainty in measurement, Geneva Switzerland, 1995.)

The coverage factor k employed was 2.

VITAMIN A

Brief description of sample preparation procedures (including sample size, internal standard, saponification conditions, extracting solvent, etc).

Calibration curve.
The trans-retinol employed for the analysis was neat chemical.
A trans-retinol solution of approximately 30 µg/g was gravimetric prepared. This solution was measured by UV-Vis spectrophotometry to evaluate the real concentration at the moment of make the calibration curve (the Lambert and Beer equation was employed: C = A/E x L).

Also the purity of this solution was evaluated injecting this dilution in the HPLC system.

The calibration curve was gravimetric prepared with the solution at different concentrations and was analyzed by HPLC.

Extraction method.
a) Approximately 5 g of sample (milk or Infant Formula) and 1.5 g of $CaCO_3$ were weighted.
b) The internal standard (trans- trans-β-apo 8´-carotenal oxime) solution was added.
c) 15 mL of water at 60°C was added and the mixture was homogenized.
d) 200 mL of THF/Methanol (50/50) was added and the mixture was homogenized for 2 min.
e) The mixture was vacuum filtered until get a filter cake. This cake was rinsed with THF/Methanol (50/50).
f) 100 mL of 10% (w/v) aqueous NaCl solution was added to the filtrate.
g) The analyte was extracted with 100 mL of 50/50 (v/v) ethyl ether/petroleum ether.
h) The extract was washed with water.
i) The organic phase was evaporated under a stream of Nitrogen (aproximately until 0.5 mL).
j) 3 mL of Ethanol were added to the solution.

Saponification of the Extract.
a) 3 mL of the extract obtained were combined with 1 mL of an antioxidant (8% w/v methanolic pyrogallol) and 0.3 ml of 40% w/v methanolic potassium hydroxide and 1.8 mL of methanol (avoid light contact).
b) This mixture was saponified and 0.1 g of ascorbic acid were added to stop the reaction.
c) The vitamins were extracted with 35 mL of 50/50 (v/v) ethyl ether/petroleum ether.
d) The phases were separated with water and the organic phase was evaporated under stream of nitrogen.
e) The extract was reconstituted with 1 ml of ethanol.
f) Inject in the HPLC.

Brief description of chromatographic method (including analytical column, mobile phase, detector, wavelengths, etc.)

The HPLC system employed to evaluate the Vitamin A (as trans-retinol) is the follow:

Ternary Varian pump model 9012.
Autosampler Bio-Rad.
Bakerbond C18 5µ 4.6 x 250 mm column.
Temperature Analysis of 29°C.
Mobile phase flow: 1.5 mL/min.

Injection volume: 20 µL.
Mobile phase Gradient:

Time (minutes)	%A	%B	%C
0	98	2	0
10	75	18	7
15	68	25	7
35	68	25	7
40	98	2	0

Where: A= ACN con 0.05% of TEA (triethyl amine)
B= 0.05 M Ammonium acetate in methanol with 0.05% TEA.
C= Ethyl acetate with 0.05% TEA.

DAD Detector at 325 nm for trans-retinol and 437 for Internal standard (oxime).

Brief description of uncertainty analysis (including source of uncertainty, degrees of freedom, type A or B, value of k, source of data [e.g., historical, calibration certificate], etc.)

The Uncertainty is expressed as an expanded uncertainty. U is calculated as $U = k\ u_c$ at the 95 % level of confidence with a coverage factor k= 2.*

The sources of uncertainty considered were:

Parameter	**Type**
Method Precision	A
Calibration curve	A
Sample preparation	B

*(International Organization for Standarization (ISO), Guide to the expression of uncertainty in measurement, Geneva Switzerland, 1995.)

Infant Formula/Milk Powder comparison (SIM.QM.P5.2)

CALCIUM: Flame Atomic Absorption Spectrometry

Brief description of sample preparation procedures (including sample size, digestion conditions, etc.)
The digestion of the sample was made using microwave digestion, sample weight 0,25 g, using 8 g of high purity nitric acid.
Digestion conditions:
Pressure of the last step: 175 psi
Total time of the digestion: 45 minutes
Temperature of the last step: 150 °C

Brief description of spectrometric method (including instrumentation, wavelengths, etc.)
We used for the measurement:
Flame Atomic Absorption Spectrometry Perkin- Elmer 5100
External calibration.
Wavelength: 422,7 nm.
Flame: Air-Acetylene

Brief description of uncertainty analysis (including source of uncertainty and its standard uncertainty, degrees of freedom, type A or B, value of K, source of data {e.g., historical, calibration certificate}, etc.)
Sample:

Source			Uncertainty	Degrees of
	value	units	*Us*	freedom
Contribution of type B evaluation				
MRC Ca	1004.00	mg/kg	2.99	inf.
Curve's standards	1.9955	mg/kg	0.0060	inf.
Calibration curve	3.126	mg/kg	0.018	15.00
Interpolation Conc. Ca in curve	3.074	uA	0.027	24.00
Sample preparation	6967.76	mg/kg	61.85	inf.
Blank correction	0.00450	uA	0.00027	5.92
Instrument	0.15500	mg/kg	0.00089	5.00
Digestion procedure	1.000	-	0.030	inf.
Control standard	0.9549	mg/kg	0.0054	inf.
Combination of type B uncertainty				
Conc. of Ca in sample	6653.21	mg/kg	211.53	inf
Contribution of type A evaluation				
Repeatability	6928.49	mg/kg	34.61	4

Conc. of Ca	6928.49	mg/kg	Degrees of freedom
u_c	214.34	mg/kg	inf
U(=k u_c)	428.69	mg/kg	k=2

Note: Source of MRC uncertainty data comes from Certificate of Analysis, the others sources of uncertainties data come from experimental data

Infant Formula/Milk Powder comparison (SIM.8.P5.2)

IRON: Inductively Coupled Plasma atomic emission Spectrometry

Brief description of sample preparation procedures (including sample size, digestion conditions, etc.)
The digestion of the sample was made using microwave digestion, sample weight 0,25 g, using 8 g of high purity nitric acid.
Digestion conditions:
Pressure of the last step: 175 psi
Total time of the digestion: 45 minutes
Temperature of the last step: 150 °C

Brief description of spectrometric method (including instrumentation, wavelengths, etc.)
We used for the measurement:
Inductively Coupled Plasma atomic emission Spectrometry IRIS Intrepid
External calibration
Measurements parameters:
Wavelength: 238,2 and 259,9 nm
Rf Power: 1100 w
Nebulizer Flow: 0,45 Lmp. Auxiliary Gas: 0,5 Lpm

Brief description of uncertainty analysis (including source of uncertainty and its standard uncertainty, degrees of freedom, type A or B, value of K, source of data {e.g., historical, calibration certificate}, etc.)

Source	value	units	Uncertainty Us	Deg. of freed
Contribution of type B evaluation				
MRC Fe	998.23	mg/kg	2.94	inf.
curve's standards	0.32466	mg/kg	0.00096	inf.
Calibration curve	0.5226	mg/kg	0.0024	15.00
blank correction	0.266	uA	0.035	9.00
Interpolation concentration Fe in curve	0.5362	mg/kg	0.0066	inf.
sample preparation	102.96	mg/kg	1.27	inf.
Instrument	10.56	uA	0.11	9.47
Digestion procedure	1.000	-	0.030	inf.
control standard	1.000	mg/kg	0.013	402.14
Combination of type B uncertainty				
C(Fe) sample	102.94	mg/kg	3.58	inf
Contribution of type A evaluation				
Reapetibility	105.07	mg/kg	0.70	9
C(fe)	105.07	mg/kg	Degrees of freedom	
u_c	3.65	mg/kg	Infinit	
U(=k u_c)	7.30	mg/kg	k=2	

Note: Source of MRC uncertainty data comes from Certificate of Analysis, the others sources of uncertainties data come from experimental data

Infant Formula/Milk Powder comparison (SIM.8.P5.2)

IRON: HR-Inductively Coupled Plasma Mass Spectrometry

Brief description of sample preparation procedures (including sample size, digestion conditions, etc.)
The digestion of the sample was made using microwave digestion, sample weight 0,25 g, using 8 g of high purity nitric acid.
Digestion conditions:
Pressure of the last step: 175 psi
Total time of the digestion: 45 minutes
Temperature of the last step: 150 °C

Brief description of spectrometric method (including instrumentation, wavelengths, etc.)
We used for the measurement:
HR- Inductively Coupled Plasma mass Spectrometry Element 1
Gallium as internal standard for calibration
Measurements parameters:
Resolution: Medium
Rf Power: 1150 w
Auxiliary gas flow: 15 L/min

Brief description of uncertainty analysis (including source of uncertainty and its standard uncertainty, degrees of freedom, type A or B, value of K, source of data {e.g., historical, calibration certificate}, etc.)

Source	value	units	Uncertainty *Us*	Degrees of freedom
Contribution of type B evaluation				
MRC Fe	998	mg/kg	2.94	inf.
MRC Ga	984	mg/kg	2.95	inf.
C(Fe) curve standards	0.01212	mg/kg	0.00004	inf.
C(Ga) curve standards	0.02467	mg/kg	0.00004	inf.
Calibration curve	0.01802	mg/kg	0.00014	5.00
Instrument Fe	164553	cps	4419	10.00
Instrument Ga	120907	cps	1103	4.00
sample preparation	101.99	mg/kg	5.10	inf.
Combination of type B uncertainty				
C(Fe) sample	101.99	mg/kg	5.10	inf.
Contribution of type A evaluation				
Reapetibility	104.79	mg/kg	0.34	9

C(fe)	104.79	mg/kg	Degrees of freedom
u_c	5.11	mg/kg	inf
U(=k u_c)	10.22	mg/kg	k=2

Note: Source of MRC uncertainty data comes from Certificate of Analysis, the others sources of uncertainties data come from experimental data

Intercomparison of Infant Formula Analyses
SIM.QM-P5

LINOLEIC ACID AND LINOLENIC ACID

Brief description of sample preparation procedures (including sample size, internal standard, hydrolysis conditions, etc.).

1. Extract the fat from the sample with hydrolysis conditions
1.1 Mix the sample, weight 2g and put it in a container Mojonnier.
1.2 Add 9ml of hot water and shake until obtain a sample well mixed
1.3 Add 1.5ml of ammonium hydroxide and mix well. Add 10ml of ethylic alcohol and 25ml of ethylic ether close the container Mojonnier and shake vigorously during 90seconds. Leave to rest aprox 1 minute
1.4 Open the container Mojonnier and add 25ml of petroleum ether close and shake vigorously during 90 seconds. Leave to rest until see a different phases in the sample.
1.5 Separate ether phase trough a Whatman paper No. 41 With sodium sulfate anhydrous, the sample put it in a flask in a constant weight
1.6 Repeat twice the extraction, with 25ml of ethylic ether and petroleum ether and the separate ether phase.
1.7 Clean the bottom of the container Mojonnier with ether phase and put it in a flask.
1.8 Put the ether phase in a rot vapor (45°C) for dry, after this put the flaks a constant weight
1.9 Weight the flask.
1.10 Calculate the % of Fat with the following formula:

$$\% \text{ Fat} = \frac{P2-P1}{Pm} \times 100$$

P1= Initial weight
P2= Final weight
Pm= Sample weight

2. Fat methilation (dried milk)
2.1 Dissolve the fat with a caustic methanolic solution (the volume depends of the amount of fat), add crystals for ebullition and concentrate until the cells of fat disappear (5-10 minutes)
2.2 Add a boron tri-flour methanol solution (the volume depends of the amount of fat) Continue the ebullition 2 minutes more or until the cells of fat disappear
2.3 Add 2.5 – 5.0ml of heptan (the volume depends of the amount of fat) and continue with the ebullition 1 minute more.
2.4 Cool the sample and add 10ml of saturate solution of sodium clorure; shake gently and add more solution of sodium clorure

Brief description of chromatographic method (including analytical column, detector, etc.).

1. Take a sample of 1-1.5ml of organic phase and put it assay tube with 0.5g of sodium sulfate anhydrous; shake well and take a sample. After this you are able to put the sample in the analytical instrument gas chromatography.

Table No.3 Chromatography conditions

Column	120-190 °C
Injector temperature	225 °C
Detector temperature	225 °C
Trap	15 °C/min
Time 1	2 minutes
Time 2	13.3 minutes
Total time	20 minutes
Range	20
ATT	16
Inyeccion volume Blanc heptane	1 µl

Brief description of uncertainty analysis (including source of uncertainty and its standard uncertainty, degrees of freedom, type A or B, value of k, source of data [e.g., historical, calibration certificate], etc.).

Intercomparison of Infant Formula Analyses
SIM.QM-P5

VITAMIN A

Brief description of sample preparation procedures (including sample size, internal standard, hydrolysis conditions, etc.).

1. Mix the sample, weight 8-10g. Add 30ml of water and 0.5g of ascorbic acid like antioxidant. Mix gently
2. Add the hydrolysis reactive, ethylic alcohol 99% pure and solution of potassium hydroxide. Shake until obtain only one sample well mixed.
3. Colocate the sample in boiling water bath shaking during 30 minutes. After this time, cool the sample an ambient temperature
4. Transfer the sample into a separate flask extraction of 500ml and extract with 250ml of ethylic ether. Wash the organic phases with portions of 100ml of distillate water until the change of color with fenoftalein
5. The sample, organic phase neutralized, dried with sodium sulfate anhydrous and put it in a flask round bottom-short neck, take it to the rot vapor for evaporation (50 °C-45°C)
6. Dissolve the sample with 5ml of HPLC Hexane and inject into the gas chromatography
7. We must use an external standard and recibe the same treatment like the dried milk sample. Weight 20mg of retinal acetate or retinal palmitate, add 50ml of HPLC hexane and dilute 1ml/25ml until we have a concentration of 7-10UI/ml.

Brief description of chromatographic method (including analytical column, detector, etc.).

1. To calculate the concentration of standard, we made a dilution 1ml/25ml with iso-propanol and read this in a Spectrophotometer, length longitude 334,325 y 310nm.[1]
2. To calculate the concentration of the sample, we used the chromatogram.

Table No.4 Conditions to measure Vitamin A

Liquid chromatography	Hewlett Packard 1050
Column	Zorbax RX-SIL,4.6 x 250mm, 5 micras
Mobile phase	3% iso-propanol in n-hexane
Flow	0.9 mL/minute
Injection volume	50 micro liters
Length longitude	326 nm
Detector	Software 32 Karat (Beckman Coulter)
Aconditionament	1 hour

Brief description of uncertainty analysis (including source of uncertainty and its standard uncertainty, degrees of freedom, type A or B, value of k, source of data [e.g., historical, calibration certificate], etc.).

Intercomparison of Infant Formula Analyses
SIM.QM-P5

VITAMIN C

Brief description of sample preparation procedures (including sample size, internal standard, hydrolysis conditions, etc.).

1. Weight 2g of sample ant put it in a volumetric flask of 25ml
2. Complete with a solution of metafosforic acid-acetic acid, gently mix and filter.
3. Take 2ml a sample in duplicate and put it in a EM Flask 25ml
4. Add 5ml of metafosforic acid-acetic acid solution and titer with a of 2,6-di-cloro-indol-fenol solution until the change of color (colorless-pink)
5. Weight 10mg of Vitamin C standard and complete 25ml with metafosforic acid-acetic acid solution
6. Take 2ml a sample in duplicate and put it in a EM flask 25ml
7. Add 5ml a metafosforic acid-acetic acid solution and titer with a of 2,6-di-cloro-indol-fenol solution until the change of color (colorless-pink)

Brief description of chromatographic method (including analytical column, detector, etc.).

Brief description of uncertainty analysis (including source of uncertainty and its standard uncertainty, degrees of freedom, type A or B, value of k, source of data [e.g., historical, calibration certificate], etc.).

Intercomparison of Infant Formula Analyses
SIM.QM-P5

IRON

Brief description of sample preparation procedures (including sample size, internal standard, hydrolysis conditions, etc.).

1. Mix the sample
2. Weight 3g of sample in duplicate and put it in volumetric flask, perfectly dried.
3. Add 25ml of desionizade water and 10ml of nitric acid concentrate
4. Colocate the sample in concentration (reflujo) for a period of 3 hours (86°C)
5. Cool the sample an ambient temperature, filter with Whatman paper No.41 and complete in a volumetric flask a volume of 50ml with desionizade water.

Brief description of chromatographic method (including analytical column, detector, etc.).

1. Read the concentration of the sample with an analytical instrument atomic absorption, Perkin Elmer 3100 with the next conditions. Table 1. Conditions
2. Use a standard curb with the following concentrations: 1,2,3,4 y 5 p.p.m.

Table No.1 Conditions

Lenght Longitude	248.3nm
Slit	0.2
Energy	60

Brief description of uncertainty analysis (including source of uncertainty and its standard uncertainty, degrees of freedom, type A or B, value of k, source of data [e.g., historical, calibration certificate], etc.).

Intercomparison of Infant Formula Analyses
SIM.QM-P5

CALCIUM

Brief description of sample preparation procedures (including sample size, internal standard, hydrolysis conditions, etc.).

1. Mix the sample
2. Weight 1g of sample in duplicate and put it in volumetric flask, perfectly dried.
3. Add 25ml of desionizade water and 10ml of nitric acid concentrate
4. Colocate the sample in concentration (reflujo) for a period of 3 hours (86°C)
5. Cool the sample an ambient temperature, filter with Whatman paper No.41 and complete in a volumetric flask a volume of 100ml with desionizade water.

Brief description of chromatographic method (including analytical column, detector, etc.).

1. Read the concentration of the sample with an analytical instrument atomic absorption, Perkin Elmer 3100 with the next conditions. Table 2. Conditions
2. Use a standard curb with the following concentrations: 1,2,3,4 y 5 p.p.m.

Table No.2 Conditions

Length Longitude	422-7nm
Slit	0.7
Energy	76

Brief description of uncertainty analysis (including source of uncertainty and its standard uncertainty, degrees of freedom, type A or B, value of k, source of data [e.g., historical, calibration certificate], etc.).

Intercomparison of Infant Formula Analyses
SIM.QM-P5

CALCIUM

Brief description of sample preparation procedures (including sample size, digestion conditions,

Three packets of milk powder were placed in plastic zipper bags and manipulated until well mixed. Two 0.5 g aliquots were taken from each bag and placed in microwave vessels along with 5 mL of HNO_3. Three packets of SRM 1846, Infant Formula, were used as a control. One 0.5 g aliquot was taken from each of the control samples and prepared along with the milk powder. All samples were digested according to the following microwave procedure. Nine blanks were also prepared along with the samples. After digestion 0.075 mL of a 10.0 mg/g In solution was added to each solution. The samples were then transferred to 30 mL plastic bottles and were diluted to 30 g with 1.5 % HNO_3.

Phase 1			Phase 2			Phase 3			Phase 4		
watts	min	watts	watts	min	watts	watts	min	watts	watts	min	watts
100	5:00	600	600	5:00	600	1000	10:00	1000	0	15:00	0

Brief description of spectrometric method (including instrumentation, wavelengths, etc.)

Calcium was determined by transferring 1 g aliquots into weighed 100 mL plastic bottles for each solution. One gram of a 10.0 mg/g In solution was added to each bottle and all aliquots were diluted to 100 g with 1.5 % HNO_3. Two 50 g aliquots were taken from each of these solutions and transferred to weighed 50 mL plastic bottles. To correct for any matrix effects caused by differences between samples and standards, standard addition was used to determine the concentration of Ca. A 0.25 g spike was taken from a solution containing 600 μg/g Ca and added to one aliquot from each sample. Calcium was determined using Inductively Coupled Plasma Optical Emission Spectrometry (ICPOES). Measurements were made at the 393.366 nm line for Ca. Two analyses were performed.

Brief description of uncertainty analysis (including source of uncertainty and its standard uncertainty, degrees of freedom, type A or B, value of k, source of data [e.g., historical, calibration certificate], etc.)

Four instrumental measurements were taken and averaged for each sample and each spiked solution. Analyte concentration is calculated by the method of standard addition.

The Type A standard uncertainty is the combined uncertainties of the sample replication and the blank replication. The Type A degrees of freedom is calculated from a combined effect of the sample and reagent blank replications. Sources of Type B uncertainty were: calibration, drift, and chemical matrix effects; primary standard; dilution of standard; and dilution of samples. The Type B standard uncertainty due to the uncertainty in the correction for calibration, drift, and chemical matrix effects is estimated to be 0.5 % of the analyte concentration, uniformly distributed. This estimate is normalized by division by $\sqrt{3}$. The Type B standard uncertainty due to the uncertainty of the primary standards was obtained from the Certificate of Analysis for the element. The Type B standard uncertainty due to the uncertainty of the dilution of standard is the combined effect of each dilution with an estimated uncertainty of ± 1mg for each dilution. This estimate is normalized by division by $\sqrt{3}$. The Type B standard uncertainty due to the uncertainty of the dilution of samples is the combined effect of each dilution with an estimated uncertainty of ± 1 mg for each dilution. This estimate is normalized by division by $\sqrt{3}$. All type B uncertainties are considered to have infinite degrees of freedom, and these uncertainties are combined. To produce the combined Type B uncertainty.

Type A and Type B uncertainty are combined and the degree of freedom of the combined uncertainty is calculated from the Satterthwaite formula. The expanded uncertainty is the product of the combined uncertainty and the coverage factor, which is the student t at 95% confidence level for the degree of freedom of the combined uncertainty.

IRON

Brief description of sample preparation procedures (including sample size, digestion conditions, etc.)

Three packets of milk powder were placed in plastic zipper bags and manipulated until well mixed. Two 0.5 g aliquots were taken from each bag and placed in microwave vessels along with 5 mL of HNO_3. Three packets of SRM 1846, Infant Formula, were used as a control. One 0.5 g aliquot was taken from each of the control samples and prepared along with the milk powder. All samples were digested according to the following microwave procedure. Nine blanks were also prepared along with the samples. After digestion 0.075 mL of a 10.0 mg/g In solution was added to each solution. The samples were then transferred to 30 mL plastic bottles and were diluted to 30 g with 1.5 % HNO_3.

Phase 1			Phase 2			Phase 3			Phase 4		
watts	min	watts	watts	min	watts	watts	min	watts	watts	min	watts
100	5:00	600	600	5:00	600	1000	10:00	1000	0	15:00	0

Brief description of spectrometric method (including instrumentation, wavelengths, etc.)

Iron was determined by transferring two 12 g aliquots from each solution into weighed 30 mL plastic bottles. To correct for any matrix effects caused by differences between samples and standards, standard addition was used to determine the concentration of Fe. A 0.1 g spike was taken from a solution containing 500 μg/g Fe and added to one aliquot from each sample. Iron was determined using Inductively Coupled Plasma Optical Emission Spectrometry (ICPOES). Measurements were made at the 259.939 nm line for Fe. Two analyses were performed.

Brief description of uncertainty analysis (including source of uncertainty and its standard uncertainty, degrees of freedom, type A or B, value of k, source of data [e.g., historical, calibration certificate], etc.)

Four instrumental measurements were taken and averaged for each sample and each spiked solution. Analyte concentration is calculated by the method of standard addition.

The Type A standard uncertainty is the combined uncertainties of the sample replication and the blank replication. The Type A degrees of freedom is calculated from a combined effect of the sample and reagent blank replications. Sources of Type B uncertainty were: calibration, drift, and chemical matrix effects; primary standard; dilution of standard; and dilution of samples. The Type B standard uncertainty due to the uncertainty in the correction for calibration, drift, and chemical matrix effects is estimated to be 0.5 % of the analyte concentration, uniformly distributed. This estimate is normalized by division by $\sqrt{3}$. The Type B standard uncertainty due to the uncertainty of the primary standards was obtained from the Certificate of Analysis for the element. The Type B standard uncertainty due to the uncertainty of the dilution of standard is the combined effect of each dilution with an estimated uncertainty of ± 1mg for each dilution. This estimate is normalized by division by $\sqrt{3}$. The Type B standard uncertainty due to the uncertainty of the dilution of samples is the combined effect of each dilution with an estimated uncertainty of ± 1 mg for each dilution. This estimate is normalized by division by $\sqrt{3}$. All type B uncertainties are considered to have infinite degrees of freedom, and these uncertainties are combined. To produce the combined Type B uncertainty.

Type A and Type B uncertainty are combined and the degree of freedom of the combined uncertainty is calculated from the Satterthwaite formula. The expanded uncertainty is the product of the combined uncertainty and the coverage factor, which is the student t at 95% confidence level for the degree of freedom of the combined uncertainty.

Intercomparison of Infant Formula Analyses
SIM.QM-P5

VITAMIN A

Brief description of sample preparation procedures (including sample size, internal standard, saponification conditions, extracting solvent, etc.)

Calibration Solutions. Solutions containing low, intermediate, and high levels (relative to expected levels of vitamins A and E in the samples) of α-, γ-, and δ-tocopherol, α-tocopheryl acetate (Eastman Kodak, Rochester, NY), and retinol, retinyl acetate, and retinyl palmitate (Sigma, St. Louis, MO) were prepared in ethanol. Concentrations of stock solutions from which calibration solutions were prepared were determined spectrophotometrically, and corrections for purity were made based on LC analysis of the stock solutions at the wavelengths at which the concentrations were determined. The calibration curves were prepared immediately following preparation of the calibration solutions. One-half mL of each calibration solution was combined with 100 mL of ethanolic *trans*-β-apo-10'-carotenal oxime, which was used as an internal standard; the resulting solution was placed in a glass insert in an autosampler vial. The autosampler, which held the samples at 15 °C, injected a 20-mL aliquot of the solution into the chromatographic system. Peak areas were used for quantitation of all analytes. Calibration curves were prepared by spreadsheet, and the y-intercept was forced through the origin.

Samples. The contents of the packet being analyzed were dumped into a 600-mL beaker, and the beaker was rotated and shaken to mix the material. Approximately 6 g of milk powder was weighed into a 400-mL beaker and 6 mL sub-boiling water (approx. 90 °C) and 500 mL *trans*-β-apo-10'-carotenal oxime internal standard solution (73.8 μg/mL) were added. The reconstituted sample was homogenized for 30 s. The homogenizer shaft was then rinsed with 35 mL 50/50 methanol/tetrahydrofuran, which was added to the reconstituted sample. To saponify the fat in the sample, 1.8 mL of a methanolic solution containing a mass fraction of 40% potassium hydroxide was added, and the mixture was stirred in a 55 °C bath for 60 min. Following saponification, approximately 0.5 g ascorbic acid was added, and the solution was mixed. Fifteen mL of an aqueous solution containing a mass fraction of 10% sodium chloride was added, and analytes were extracted into two 15-mL portions of 50/50 diethyl ether/hexane using a separatory funnel. The organic phase was washed with water, and the organic solvents were evaporated to approximately 100 mL under a stream of nitrogen. Samples were combined with approximately 1 mL of a 50/50 mixture of ethyl acetate and ethanol containing 30 mg/mL butylated hydroxytoluene (BHT, an antioxidant); 10 mL of the resulting solution was injected into the LC system.

Brief description of chromatographic method (including analytical column, mobile phase, detector, wavelengths, etc.)

A 25-cm Bakerbond C_{18} analytical column (Lot No. F48082-06, J.T. Baker, Phillipsburg, NJ) was held at 29 °C by a column oven. Solvent A was acetonitrile, solvent B was methanol containing 0.05 mol/L ammonium acetate, and solvent C was ethyl acetate. Each of the three solvents contained a volume fraction of 0.05% triethylamine. The method consisted of two

linear gradients and an isocratic component. The first gradient began with 98% A/2% B and went to 75% A/18% B/7% C in 10 min. A second linear gradient ran from this composition to 68% A/25% B/7% C in 5 min. This composition was held for 23 min longer, then the system was returned to initial conditions (98% A/2% B) over 5 min and re-equilibrated for 5 min. The flow rate was 1.5 mL/min. The stainless steel frits inserted in the column by the manufacturer were replaced with "biocompatible" frits. A programmable UV/visible absorbance detector with a tungsten lamp was used for measurement of the retinoids and the internal standard. The wavelength was held at 325 nm to measure retinol and retinyl acetate, and then was changed to 450 nm at 4.5 min to measure the internal standard; it was then changed to 292 nm to measure the tocopherols, and returned to 325 nm to measure retinyl palmitate. A fluorescence spectrometer was also used to measure the tocopherols using an excitation wavelength of 295 nm and an emission wavelength of 335 nm. Signals from both detectors were recorded simultaneously by the data system.

Brief description of uncertainty analysis (including source of uncertainty and its standard uncertainty, degrees of freedom, type A or B, value of k, source of data [e.g., historical, calibration certificate], etc.)

Uncertainty Components, milk powder	
Measurement of Samples, std uncert	0.1093
Measurement of Calib Stds, std uncert	0.0019
Measurement of Stock Soln, std uncert	0.0000079
Combined Standard Uncertainty	0.1094
Effective degrees of freedom	5.0031
k (from t-distribution)	2.5706
Expanded Uncertainty	0.2811
Expanded Uncertainty as %	6.63%

Uncertainty Components, SRM 1846	
Measurement of Samples, std uncert	0.0898
Measurement of Calib Stds, std uncert	0.0016
Measurement of Stock Soln, std uncert	0.0000065
Combined Standard Uncertainty	0.0898
Effective degrees of freedom	2.0012
k (from t-distribution)	4.3027
Expanded Uncertainty	0.3863
Expanded Uncertainty as %	11.05%

Intercomparison of Infant Formula Analyses
SIM.QM-P5

VITAMIN C

Brief description of sample preparation procedures (including sample size, internal standard, saponification conditions, extracting solvent, etc.)

Sampling Procedure. Approximately 2-g samples were weighed into 50-mL Teflon centrifuge tubes on a top loading balance (to 1 mg). Three sets of analyses were performed. In the first set only a duplicate sample of a single package of SRM 1846 was analyzed in duplicate (total 4 analyses, 2/sample). In the second and third sets duplicate samples of three CENAM samples and one SRM 1846, infant formula, package were analyzed in duplicate (total 16 analyses, 2/sample).

Sample Preparation. Samples (1.7 g - 2.1 g) were weighed to the nearest mg into 50-mL Teflon centrifuge tubes. Twenty mL of an aqueous solution of containing K_2HPO_4 (0.05 mol/L) and dithiothreitol (DTT, 1 g/L) was weighed into the centrifuge tube and the material was mixed and allowed to dissolve. After all samples had dissolved 5 mL of metaphosphoric acid (MPA, 50 g/100 mL) and 10 mL of acetonitrile were weighed in sequentially. The samples were mixed and then centrifuged at 1500 x G for 15 min at 5 °C in an Eppendorf centrifuge, model 5403. Four mL were removed from the clear layer. This was further diluted with extraction solvent 1:75 (100 µL to 7.500 mL) or, in the case of the milk samples in set. 3, 1:37.5 (100 µL to 3.750 mL) with a Micromedic pump in the diluting mode. The diluted samples were mixed, transferred to washed autosampler vials and analyzed by HPLC.

Calibration and Standardization. The instrument was calibrated with three external standards which bracketed the ascorbic acid (AA) concentrations of the samples. These standards were made up in water:acetonitrile 1:3 containing DTT (1 mg/mL). The stock contained an AA concentration of approximately 0.5 mmol/L (approx. 10 mg/mL, weighed to 0.1 mg). The external standards were prepared from this stock solution by making dilutions of 1:100, 1:200 and 1:400. These values were used to calculate the final concentrations of the AA in the powdered samples.

Brief description of chromatographic method (including analytical column, mobile phase, detector, wavelengths, etc.)

The HPLC analysis was performed on a Capcel Pak NH2 column (4.6 mm x 250 mm) equilibrated at 40 °C using a 25 µL sample. The electrochemical detector was set at 700 mV. Chromeleon software was used to integrate the chromatograms.

The HPLC mobile phase was formulated as follows:

0.680 g KH_2PO_4
200 mL water

800 mL acetonitrile, HPLC grade
7.5 mL phosphoric acid.

Brief description of uncertainty analysis (including source of uncertainty and its standard uncertainty, degrees of freedom, type A or B, value of k, source of data [e.g., historical, calibration certificate], etc.)

Uncertainty Components, milk powder	
Measurement of Samples, standard uncertainty	8.5061
Combined Standard Uncertainty	8.5061
Effective degrees of freedom	5.0000
k (from t-distribution)	2.5706
Expanded Uncertainty	21.8657
Expanded Uncertainty as %	7.52%
Uncertainty Components, SRM 1846	
Measurement of Samples, standard uncertainty	35.0773
Combined Standard Uncertainty	35.0773
Effective degrees of freedom	2.0000
k (from t-distribution)	4.3027
Expanded Uncertainty	150.9255
Expanded Uncertainty as %	13.14%

Intercomparison of Infant Formula Analyses
SIM.QM-P5

LINOLEIC ACID AND LINOLENIC ACID

Brief description of sample preparation procedures (including sample size, internal standard, hydrolysis conditions, etc.)

Sampling and Extraction. A set of three samples of each material (SRM 1846 and CENAM milk powder) was prepared for extractable fat determination. The sample mass (ca. 0.9 g) was determined by weighing to ± 0.1 mg, and then the sample was dispersed on 2 g of Hydromatrix. The material was moistened with 0.4 mL deionized water, and then mixed into the dispersant and poured into the extraction cell. The samples were extracted with hexane: acetone 4:1 (v/v).

The samples were extracted by consecutive pressurized fluid extraction (Dionex ASE), using hexane:acetone (4:1). Each extraction consisted of three cycles. Each cycle was defined with an oven heat time of 6.0 minutes to 125 °C, a 5 minute extraction at 1500 psig, a 100% (of cell volume) solvent flush, and a 60 second nitrogen purge. Each extraction was followed by blowing down the samples under nitrogen in a water bath at 35 – 40 °C, and drying in a gravity convection oven at 100 - 101 °C for 30 min. The extracts were allowed to cool in a desiccator for at least 20 min prior to weighing. This was repeated until gravimetric analysis of the combined dried extracts showed no further increase in mass, compared to the previous extraction/drying cycle. These partially dried extracts still contained some residual solvent. Interim dried sample masses increased until the fat content of the sample was fully exhausted, and then dropped quickly as no fresh extractable fat was present during the drying cycle. SRM 1846 was found to be exhausted after three extractions, whereas the CENAM milk powder required four extractions. The final extracts were then subjected to repeated drying cycles of 30 min at 100 – 102 °C until the mass loss between oven cycles was less than 0.05%. This occurred after a total drying time of two hours for both materials.

For fatty acid analysis, three duplicate samples of SRM 1846 were run in two sets. For the CENAM milk powder, three individual packets of material were opened, mixed and sampled two times each. All six CENAM milk powder samples were run in one set to allow the assessment of sample homogeneity. The fatty acid samples were prepared and extracted as for the extractable fat samples, except that the materials were only partially blown down between consecutive ASE extractions. Each set included one additional sample that was analyzed for extractable fat to ensure complete extraction.

Sample Preparation. After ASE, the combined extracts were diluted to known volume (50 ml), and a 20% aliquot was combined with internal standard (C 13:0 FAME) solution. Internal standard solution, prepared in hexane, was added to a series of 16 x 125 mm culture tubes. 10 mL of the diluted extract was accurately transferred by volumetric pipette into each of the spiked tubes. 1.5 mL of 0.5 mol/L NaOH (in methanol) was added, blanketed with N_2, capped, mixed, and then heated in a dry bath at 100 °C for 5 min. This mixture was cooled, 2.0 mL of 12%

boron trifluoride in methanol was added, blanketed with N_2, capped, mixed, and then heated in a dry bath at 100 °C for 30 min. This two-step process produces a higher level of conversion than either catalyst alone. The mixture was cooled to 40 °C, 2.0 mL of hexane with 50 mg/L 2,6-di-tert-butyl-para-cresol CAS# 128-37-0 (BHT) was added, blanketed with N_2, capped, mixed for 0.5 min, while still warm. 5.0 mL of saturated NaCl(aq.) was immediately added, blanketed with N_2, capped, mixed for 1.0 min., and allowed to cool to room temperature. The hexane layer was transferred to a 13 x 100 mm culture tube, blanketed with N_2, and capped. This material was analyzed by gas chromatography at this dilution.

Brief description of chromatographic method (including analytical column, detector, etc.)

Two microliter aliquots of the extracts were analyzed by split injection at a split ratio of 40:1. A Supelco SP-2340 column [100% poly(biscyanopropylsiloxane)], 0.32 mm i.d. x 30 m long x 0.20 μm film, was used. The GC oven temperature was programmed from 150 °C (15 min) to 230 °C at 4 °C min^{-1}, then held at 230 °C for 15 min. Flow-programmed helium carrier was used at 25 cm sec^{-1} initially, increased to 30 cm sec^{-1} at 15 min, 35 cm sec^{-1} at 30 min, and finally to 40 cm sec^{-1} at 40 min. Data acquisition and analysis was done on a HP Chemstation data system. A mass-based calibration curve was applied to each component, bracketing the preliminary measured values. Component concentrations in the standard mixture were based preliminary values, and dilutions were prepared bracketing the estimated component concentration. The stock standard solutions were brought to volume with hexane/BHT as an antioxidant. The diluent included the internal standard (C13:0), which was kept at the same concentration in the working standard mixtures. Sample data was corrected for the reference peak (C18:0) retention time, internal standard (C13:0) response, and the observed relative response factor for each component in the standard mixture.

Brief description of uncertainty analysis (including source of uncertainty and its standard uncertainty, degrees of freedom, type A or B, value of k, source of data [e.g., historical, calibration certificate], etc.)

Uncertainty Components, milk powder	Linoleic Acid	Linolenic Acid
Measurement of Samples, standard uncertainty	0.0817	0.0035
Combined Standard Uncertainty	0.0817	0.0035
Effective degrees of freedom	5.0000	5.0000
k (from t-distribution)	2.5706	2.5706
Expanded Uncertainty	0.2100	0.0089
Expanded Uncertainty as %	4.03%	4.81%
Uncertainty Components, SRM 1846		
Measurement of Samples, standard uncertainty	0.0398	0.0004
Combined Standard Uncertainty	0.0398	0.0004
Effective degrees of freedom	2.0000	2.0000
k (from t-distribution)	4.3027	4.3027
Expanded Uncertainty	0.1710	0.0019
Expanded Uncertainty as %	4.19%	1.94%

Intercomparison of Infant Formula Analyses
SIM.QM-P5

LABORATORY:

VITAMIN A

Brief description of sample preparation procedures (including sample size, internal standard, saponification conditions, extracting solvent, etc.)

Saponify overnight at room temperature, 1,00g of sample + 5 ml Sodium Ascorbate (200g/l) + 6 ml OHK 50% in Methanol + 20 ml Ethanol. Add 40 ml of water and extract three times with 30 ml of Hexane. Wash the hexane layer with water to neutral pH, filter through anhydrous Sodium Sulfate. Make up to volume with hexane in a 100 ml volumetric flask. Inject this solution in HPLC.
Standard (USP Std is used).
The standard is saponified and extracted in the same way as the sample.
The working standard concentration is 0.5 IU/ml in hexane.
Conversion factor: 1 UI = 0,000344 mg Vit. A (acetate)

Brief description of chromatographic method (including analytical column, mobile phase, detector, wavelengths, etc).

Column LiChrospher Si-60 (5 μm) 250 mm x 4 mm
Fluorescence Detector λ Ex 325 nm
λ Em 480 nm
Injection volume: 20 μl
Mobile phase: Hexane – Isopropyl-alcohol (99-1)
Flow: 1,5 ml/min

Brief description of uncertainty analysis (including source of uncertainty and its standard uncertainty, degrees of freedom, type A or B, value of k, source of data [e.g. historical, calibration certificate], etc).

The available standard for Vitamin A is not certified with an uncertainty value. For vitamin A, USP standard has been used. For the determination of vitamin A the USP standard must be treated following several steps (digestion, extraction, etc.) and for that reason the uncertainty of the standard is unknown. We developed a procedure to evaluate the uncertainty based on the differences between the measured and certified values of SRM 1846. The difference has been combined (assuming rectangular distribution) with the statistic contribution (repeatability)

VITAMIN C

Brief description of sample preparation procedures (including sample size, internal standard, saponification conditions, extracting solvent, etc.)

Pulverize sample by gentle grinding, add HPO3-HOAc+HSO4+EDTA solution, homogeneize and diluting to measure volumen.
Centrifugue the solution and then filter it.
Transfer three 2.0 ml aliquots ascorbic acid std. solution to each of three 50 ml erlenmeyers containing 5.0 ml HPO3-HOAc+HSO4+EDTA soln. Titrating rapidly with indophenol until light but distinct rose pink persist 5 sec.
Similarly titr. 3 blanks composed of 7.0 ml HPO3-HOAc+HSO4+EDTA solution, plus volume H2= equal to volume indophenol soln, used in direct titrations.
Titrating 3 sample aliquots each containing 2 mg ascorbic acid with indophenol until ligth but distinct rose pink persist 5 sec.
Standard (L<+> ascorbic acid Merck is used).
The working standard concentration is 1 mg/ml.

Brief description of uncertainty analysis (including source of uncertainty and its standard uncertainty, degrees of freedom, type A or B, value of k, source of data [e.g. historical, calibration certificate], etc).

See Vitamin A

LINOLEIC ACID AND LINOLENIC ACID

Brief description of sample preparation procedures (including sample size, internal standard, hydrolysis conditions, etc.)

- *Methylating reagent: 4% H_2SO_4 in methanol*
- *Procedure:*
  - *Weight out approximately 100 mg of sample in a glass tube (13x10 mm) with teflon lined caps.*
  - *Add 5 ml of sulfuric acid (methylating reagent) to the test tube and mix by vortex for 20 seconds. Cap tube tightly and place in water bath at 100°C for one hour.*
  - *Vortex tube every 20 minutes.*
  - *Cool to room temperature, add 2 ml de-ionized water and 1 ml hexane.*
  - *Vortex, centrifuge, and inject 1 µl of upper layer.*
- *Fat in dried milk: AOAC (1996) Official Method 932. 06*

Brief description of chromatographic method (including analytical column, detector, etc).

- *Temperature programmable gas chromatograph equipped with a FID and manual injection system.*
- *Capillary column: SUPELCO, SP - 233 D (30 m x 0,25 mm x 0,2 μm film thickness).*
- *Computer assisted data acquisition and reduction system.*
- *Chromatography grade gases: He, air, H_2.*
- *Standard SUPELCO – F.A.M.E. Mix rapeseed oil Catalogue N° 07756 – Lot N° LA – 90776.*
- *Calculations:*

  With 18:2 (linolenic acid) and 18:3 (linolenic acid) A% values from chromatogram, fat content and the conversion factor we calculate the concentration values for fatty acids (as triglycerides).

Brief description of uncertainty analysis (including source of uncertainty and its standard uncertainty, degrees of freedom, type A or B, value of k, source of data [e.g. historical, calibration certificate], etc).

See Vitamin A

Intercomparison of Infant Formula Analyses
SIM.QM-P5

LABORATORY:

VITAMIN A

Brief description of sample preparation procedures (including sample size, digestion conditions, etc.)

- *Saponification and extraction of 20 g of dried sample*
- *Cuantification with external standard of retinol*
- *Determination by HPLC*
- *International IDF Provisional standard: 142:1990*

Brief description of spectrometric method (including instrumentation, wavelengths, etc).

- *Detector: UV, 325 nm*
- *Analytical column: C18*
- *Mobile phase: Methanol: Water (90+10)*
- *Temperature: ambient*
- *Injection Volume: 20 µl*

Brief description of uncertainty analysis (including source of uncertainty and its standard uncertainty, degrees of freedom, type A or B, value of k, source of data [e.g. historical, calibration certificate], etc).

See Vitamin A, LAB: 23

LINOLEIC ACID AND LINOLENIC ACID

Brief description of sample preparation procedures (including sample size, digestion conditions, etc.)

- *Weight 5-10 g of sample and then reconstituted with destilled water*
- *Fat extraction with Triton x – 100 solution in an oven at 95 °C – 100 °C*
- *Weight 45 mg of fat and then esterified with sodium methanol solution to form fatty acids methyl esters*
- *Cuantification with external standard of linoleic and linolenic acid*
- *Determination of fat content by Gerber Method*

Brief description of spectrometric method (including instrumentation, wavelengths, etc).

Intercomparison of Infant Formula Analyses
SIM.QM-P5

LABORATORY:

VITAMIN A

Brief description of sample preparation procedures (including sample size, digestion conditions, etc.)

- *Method FIL – IDF 142: 1990*
- *Saponification and extraction of 20 g of dried sample*
- *Cuantification with external standard of retinol*
- *Determination by HPLC*

Brief description of spectrometric method (including instrumentation, wavelengths, etc).

- *Detector: UV, 325 nm*
- *Analytical column: C18, 25 cm x 4,6 mm x 5u*
- *Mobile phase: Methanol: Water (90+10)*
- *Flow: 2 ml / min*

Brief description of uncertainty analysis (including source of uncertainty and its standard uncertainty, degrees of freedom, type A or B, value of k, source of data [e.g. historical, calibration certificate], etc).

See VITAMIN A, LAB: 23

VITAMIN C

Brief description of sample preparation procedures (including sample size, digestion conditions, etc.)

- *Method AOAC 985.33*
- *Weight 7 g of dried sample*
- *Dissolve with EDTA: Metaphosphoric acid (1:1)*
- *Determination by titration with indophenol*

Brief description of uncertainty analysis (including source of uncertainty and its standard uncertainty, degrees of freedom, type A or B, value of k, source of data [e.g. historical, calibration certificate], etc).

See VITAMIN A, LAB: 23

CALCIUM

Brief description of sample preparation procedures (including sample size, digestion conditions, etc.)

- *2 g of dried sample + 20 - 25 ml distilled water, shacked gently*
- *Rest 1 hour shacking for time to time*
- *Adjust pH 12.5 – 13 with NaOH*
- *Titrate with EDTA 0,01 M*

Brief description of uncertainty analysis (including source of uncertainty and its standard uncertainty, degrees of freedom, type A or B, value of k, source of data [e.g. historical, calibration certificate], etc).

Symbol	Source	Quantity	Distribution	Divisor	c_i	u_i	Degrees of freedom
V	Burette Volume	0.02 ml	Rectangular	3	200	2,3	
N	Norm. EDTA	0,0001 meq/ml	Normal	1	800000	80	2
PM	Atomic weight	0,01 mg/meq	Rectangular	3	200	1,15	
m	Mass	0,0001g	Normal	1	2000	0,2	
a	TIPO A	15 mg/g	Normal	1	1	15	
uc	Combined uncertainty		Normal			99	
U	Expanded uncertainty		K = 2			198	

Calculation:

ug Ca / g de powder milk = [(Vg -Vb) . N .PM. 1000] / sample mass (g)

Vg: volume of EDTA used for the sample (ml)

Vb: volume of EDTA used for the blank (ml)

N: concentration of EDTA solution

PM: Calcium atomic weight.

Brief description of uncertainty analysis (including source of uncertainty and its standard uncertainty, degrees of freedom, type A or B, value of k, source of data [e.g. historical, calibration certificate], etc).

See VITAMIN A, LAB: 23

CALCIUM

Brief description of sample preparation procedures (including sample size, digestion conditions, etc.)

- *2 g of dried sample + 20 - 25 ml distilled water, shacked gently*
- *Rest 1 hour shacking for time to time*
- *Adjust pH 12.5 – 13 with NaOH*
- *Titrate with EDTA 0,01 M*

Brief description of uncertainty analysis (including source of uncertainty and its standard uncertainty, degrees of freedom, type A or B, value of k, source of data [e.g. historical, calibration certificate], etc).

Symbol	Source	Quantity	Distribution	Divisor	c_i	u_i	Degrees of freedom
V	Burette Volume	0.02 ml	Rectangular	3	200	2,3	
N	Norm. EDTA	0,0001 meq/ml	Normal	1	800000	80	2
PM	Atomic weight	0,01 mg/meq	Rectangular	3	200	1,15	
m	Mass	0,0001g	Normal	1	2000	0,2	
a	TIPO A	15 mg/g	Normal	1	1	15	
uc	Combined uncertainty		Normal			99	
U	Expanded uncertainty		K = 2			198	

Calculation:

ug Ca / g de powder milk = [(Vg -Vb) . N .PM. 1000] / sample mass (g)

Vg: volume of EDTA used for the sample (ml)

Vb: volume of EDTA used for the blank (ml)

N: concentration of EDTA solution

PM: Calcium atomic weight.