

# Farming, well water consumption, rural living, and pesticide exposure in early life as the risk factors for Parkinson disease in Iğdır province

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## ABSTRACT

**الأهداف:** الكشف المبكر عن مرض باركنسون (الرعاش)، وعن عوامل الخطر في الحياة الزراعية، وفي مياه الآبار المستعملة، وكذلك عوامل الخطر في الحياة الريفية وفي مبيدات الآفات الزراعية التي تؤدي إلى الإصابة بهذا المرض وذلك للأشخاص الذين يعيشون في ولاية إغدير في الجمهورية التركية.

**المنهجية:** ذكرت في البحث 72 من الأشخاص المرضى بمرض باركنسون (الرعاش) الذين عولجوا بمشفى إغدير الحكومي في قسم الأمراض العصبية، وأجريت البحث على هؤلاء الأشخاص من خلال إلقاء عليهم بعض الأسئلة المتعلقة بحياتهم القروية واستعمالهم مياه الآبار، وانقسمت أمراضهم فيما يتعلق باستهلاك المياه إلى قسمين: قسم كانت أمراضهم بسبب استهلاك ماء البئر، وقسم كانت أمراضهم بسبب استهلاك مياه المدن. وبعد تحليل مادة (السلفات، النترات) في مياه الآبار ومياه المدن ست مرات تبين أن في كلا المائتين مستوى معين من النترات والسلفات.

**النتائج:** كان مرض 49 شخصاً من 72 شخص بسبب تعرضه وشربه من مياه الآبار، بينما الـ 23 (31.9%) شخص الآخرين لم يتعرضوا أو يشربوا من مياه الآبار. إذاً هناك علاقة بين مرض باركنسون (الرعاش) وبين الحياة الريفية، والولادة في الريف، واستعمال ماء البئر، من خلال ما تبين خلال السكن في ولاية إغدير.

**الخلاصة:** هذا أول بحث في الجمهورية التركية يجعل علاقة وارتباط بين مرض باركنسون (الرعاش) واستعمال ماء البئر.

**Objectives:** To investigate potential risk factors for Parkinson's disease (PD) in elderly individuals rural living in Turkey.

**Methods:** In total, 72 consecutive elderly Parkinson disease patients referred to the Neurology Clinic, Iğdır State Hospital, Iğdır, Turkey were included in the study. A structured questionnaire comprising questions on history of pastoral living, pit water consumption, and exposure to ionizing radiation

and pesticides was administered to the patients. The patients were divided into 2 groups on the basis of water consumption: well water consumption group and city network consumption group.

**Results:** Of 72 patients with PD, 49 (68.1%) exposed to well water while 23 (31.9%) did not exposed to well water. The average duration of well water consumption was 20 (standard deviation 6) years ( $p<0.01$ ) in group 1. Nitrate, sulfate and heavy metal levels were significantly higher in well water than in city network water ( $p<0.05$ ).

**Conclusion:** Consumption of well water containing heavy metals and nitrates in early life may contribute to the etiology of Parkinson disease in elderly individuals in Iğdır province of Turkey.

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Parkinson disease (PD) is a major cause of morbidity in senior individuals. The major pathophysiology of this disorder entails death of dopaminergic neurons and degeneration of neuronal projections to the basal ganglia. The etiology of the disease is multifactorial, and interactions between genetic and environmental factors play a critical role in the development of the disorder.<sup>1</sup> Reportedly, well water consumption, farming, smoking, pesticides, rural living, diet, and coffee and alcohol consumption as well as ionizing radiation, head trauma,

and infections are the potential environmental risk factors for PD.<sup>2-4</sup> Studies<sup>1,2,3,4</sup> have reported controversial results, but for the most part, they have confirmed the association between environmental factors and PD.<sup>2</sup> In most of the studies, smoking has been found to be negatively associated with PD.<sup>3</sup>

In rural areas, particularly among farmers, consumption of well water contaminated with nitrates, phosphates, and heavy metals that are components of chemical fertilizers and pesticides is a potentially important risk factor for informed consent was obtained from all the patients. A structured questionnaire comprising 10 questions was administered to PD patients and controls to obtain information about place of birth, residence, occupation, history of head trauma, family history of PD and essential tremor, rural living, history of well water consumption, PD.<sup>2,3</sup> Iğdir province is a rural area in eastern Turkey where agriculture is the main source of income. PD is very common in Iğdir province, and Metsamor nuclear reactor is located at a distance of approximately 16 km from this province.<sup>4</sup> We aimed to investigate farming, well water consumption, rural living, and pesticide exposure as potential risk factors for PD in elderly individuals of Iğdir province through a case-control study and through chemical analysis of the well water for nitrate, phosphate, and heavy metal levels.

**Methods.** This is prospective controlled the study. In total, 72 consecutive patients with PD confirmed by a neurologist who were referred to the Iğdir State Hospital Neurology Clinic, Iğdir, Turkey, between June 24, 2016, and October 1, 2016, were enrolled in this study. Ethics Committee approval was received from Van Yuzuncu Yıl University Clinical Applications Ethics Committee (Decision number: 21.09.2017/06). All procedures were carried out according to the ethical rules specified in the Helsinki Declaration revised at 2000. Inclusion criteria were presence of 2 or more of the following clinical properties of PD: rigidity, resting tremor, and bradykinesia; chronic progression of symptoms; and absence of autonomic, amyotrophic, pyramidal, cerebellar, and supranuclear oculomotor signs. Furthermore, only those individuals who had been living in Iğdir before the age of 30 were included. Exclusion criteria were drug-induced

parkinsonian symptoms or parkinsonian symptoms due to encephalitis, brain trauma, Huntington's or Wilson's disease, brain tumor, multiple cerebral infarcts, normal pressure hydrocephalus, and carbon monoxide intoxication. exposure to ionizing radiation, exposure to pesticides, and cigarette smoking. All exposures were self-reported. Well water consumption was considered before the age of 30. Based on water consumption, patients were divided into 2 groups: (1) well water consumption and (2) city network water consumption.

**Measurement of water analysis.** Nitrate, sulfate, fluorine, chlorine, bromine, beryllium, iron, magnesium, phosphate, chromium, cadmium, manganese, cobalt, nickel, arsenic, selenium, strontium, molybdenum, tin, titanium, vanadium, silver, thallium, lithium, and heavy metal levels were measured in both well water and city network water samples. Each parameter was spectrophotometrically analyzed 6 times using ICP-MS Thermo Scientific XSERIES 2 instrument. Water samples are filtered through 0.22 $\mu$  filters to remove potential suspensions. Then, samples are pulled into the ICP-MS instrument for 40 seconds. After nebulization and the elements are carried into Ar plasma for excitation. Detection of the elements are performed via mass spectrometer. Each analysis is repeated three times. The results were expressed in mg/L.

**Statistical analysis.** The Statistical Package for the Social Sciences package program (version 21) was used for statistical evaluations. The characteristics were presented as mean, standard deviation (SD), minimum and maximum values, and percentages. Mann-Whitney U-test was enforced to compare the groups. Chi-square test was enforced to determine the relationship between categorical variables. In addition, Z-test was used for

**Table 1 -** Demographics of the study groups.

Gender Characteristics	Male	Female	Total
	n (%)		
<i>*Network water</i>			
Min. -Max.	53-85		
Age (year) Mean $\pm$ SD	69.60 $\pm$ 9.583		
n(%)	16 (69.6)	7 (30.4)	23 (100)
Total Percentage (%)	(22.2)	(1.4)	(31.9)
<i>Well water</i>			
Min. -Max.	23-83		
Age (year) Mean $\pm$ SD	67.62 $\pm$ 12.422		
n(%)	30 (61.2)	19 (38.8)	49 (100)
Total Percentage (%)	(41.7)	(26.4)	(68.1)
<b>Total n(%)</b>	46 (63.9)	26 (36.1)	72 (100)
Min. -Max.	23-85		

\*P-values=0.454, Min - minimum, Max - maximum

**Disclosure.** Authors have no conflict of interests, and the work was not supported or funded by any drug company.

**Table 2 -** Comparison of heavy metal levels in well water and city network water (µg/L).

		n	Mean	Std. Dev.	95% Confidence Interval for Mean		Minimum	Max.	P-values
					Lower Bound	Upper Bound			
Li	Network water	3	13.8633	.67122	12.1959	15.5307	13.17	14.51	.033
	Well water	3	16.5367	1.27962	13.3579	19.7154	15.06	17.32	
	Total	6	15.2000	1.72604	13.3886	17.0114	13.17	17.32	
Be	Network water	3	.6817	.19741	0.1913	1.1721	.50	.89	.001
	Well water	3	-.3177	.05604	-0.4546	-0.1785	-.35	-.25	
	Total	6	.1820	.56253	-0.4083	0.7723	-.35	.89	
Mg	Network water	3	38356.6667	812.97806	363.37.1112	40376.2161	37430.00	38950.00	.004
	Well water	3	46000.0000	2138.24695	40688.3001	51311.6999	43550.00	47490.00	
	Total	6	42178.3333	4429.37656	37529.9877	46826.6790	37430.00	47490.00	
P	Network water	3	-14.4133	2.00056	-19.3830	-9.4437	-16.71	-13.05	.076
	Well water	3	-9.2710	3.17157	-17.1496	-1.3924	-12.91	-7.10	
	Total	6	-11.8422	3.68205	-15.7062	-7.9781	-16.71	-7.10	
Cr	Network water	3	6.7373	.11261	6.4576	7.0171	6.62	6.85	.001
	Well water	3	4.9013	.22542	4.3414	5.4613	4.70	5.15	
	Total	6	5.8193	1.01817	4.7508	6.8878	4.70	6.85	
Mn	Network water	3	.9437	.54979	1.2246	3.9561	2.26	3.23	.027
	Well water	3	2.5903	.62906	-0.6190	2.5063	.51	1.67	
	Total	6	1.7670	1.04530	0.6700	2.8640	.51	3.23	
Fe	Network water	3	19.1100	2.30671	13.3798	24.8402	16.98	21.56	.397
	Well water	3	22.3767	5.50390	8.7042	36.0491	16.31	27.05	
	Total	6	20.7433	4.17694	16.3599	25.1268	16.31	27.05	
Co	Network water	3	.6363	.18413	0.1789	1.0937	.49	.84	.010
	Well water	3	.1050	.07721	-0.0868	0.2968	.02	.15	
	Total	6	.3707	.31724	0.0377	0.7036	.02	.84	
Ni	Network water	3	1.9400	1.35064	-1.4152	5.2952	1.03	3.49	.908
	Well water	3	2.0537	.86208	-0.0879	4.1952	1.35	3.02	
	Total	6	1.9968	1.01530	0.9313	3.0623	1.03	3.49	
As	Network Water	3	12.2733	2.62348	5.7563	18.7904	10.65	15.30	.257
	Well water	3	10.0153	1.36587	6.6223	13.4084	9.15	11.59	
	Total	6	11.1443	2.24251	8.7910	13.4977	9.15	15.30	
Se	Network water	3	3.8573	6.96009	-13.4325	21.1472	-.79	11.86	.280
	Well water	3	-1.4750	2.51352	-7.7189	4.7689	-2.97	1.43	
	Total	6	1.1912	5.51674	-4.5983	6.9806	-2.97	11.86	
Sr	Network water	3	706.8667	16.70968	665.3575	748.3758	688.00	719.80	.006
	Well water	3	838.1667	39.04617	741.1706	935.1627	793.80	867.30	
	Total	6	772.5167	76.76870	691.9529	853.0805	688.00	867.30	
Mo	Network water	3	-9.7567	.68743	-11.4643	-8.0490	-10.53	-9.22	.004
	Well water	3	-12.2133	.17616	-12.6509	-11.7757	-12.40	-12.05	
	Total	6	-10.9850	1.41845	-12.4736	-9.4964	-12.40	-9.22	
Cd	Network water	3	-2.2207	.82416	-4.2680	-0.1733	-2.90	-1.31	.006
	Well water	3	-5.2780	.58929	-6.7419	-3.8141	-5.89	-4.72	
	Total	6	-3.7493	1.79298	-5.6310	-1.8677	-5.89	-1.31	
Sn	Network water	3	1.1050	.26491	0.4469	1.7631	.85	1.38	.001
	Well water	3	-.4310	.13583	-0.7684	-0.0936	-.56	-.29	
	Total	6	.3370	.86211	-0.5677	1.2417	-.56	1.38	

Sn - stannic, Cd - cadmium, Mo - molybdenum, Sr - strontium, Se - selenium, As - arsenic, Ni - nickel, Co - cobalt, Fe - iron, Mn - manganese, Cr - chromium, Li - lithium, Be - beryllium, Mg - magnesium, P - phosphorus

**Table 3 -** Comparison of nitrate and sulfate levels between groups (µg/L).

Groups	N	Mean	Std. Dev.	95% Confidence Interval for Mean		Min.	Max.	P-values
				Lower Bound	Upper Bound			
<i>F</i>								
Well water	2	.16465	.006576	0.10557	0.22373	.160	.169	.070
Network water	2	.14360	.005091	0.09786	0.18934	.140	.147	
Total	4	.15413	.013067	0.13333	0.17492	.140	.169	
<i>Cl</i>								
Well water	2	46.17500	.247487	43.95141	48.39859	46.000	46.350	.028
Network water	2	47.19185	.002616	47.16834	47.21536	47.190	47.194	
Total	4	46.68343	.604219	45.72198	47.64487	46.000	47.194	
Nitrate								
Well water	2	7.03330	.004667	6.99137	7.07523	7.030	7.037	.001
Network water	2	6.60365	.005162	6.55727	6.65003	6.600	6.607	
Total	4	6.81848	.248091	6.42371	7.21324	6.600	7.037	
Sulfate								
Well water	2	84.48475	.006718	84.42440	84.54510	84.480	84.490	.001
Network water	2	82.55360	.005091	82.50786	82.59934	82.550	82.557	
Total	4	83.51917	1.114961	81.74502	85.29333	82.550	84.490	
<i>Phosphate</i>								
Well water	2	.00415	.000212	0.00224	0.00606	.004	.004	.028
Network water	2	.00310	.000141	0.00183	0.00437	.003	.003	
Total	4	0.00363	0.000624	0.00263	0.00462	0.003	0.004	

comparison of proportions. Results with a *p*-value of 0.05 or less were noted significant.

**Results.** Of the 72 consecutive PD patients in Iğdir province included in this trial, 49 (68.1%) exposed to well water while 23 (31.9%) did not exposed to well water and 26 (36.1%) were female and 46 (63.9%) were male. The average age was 67.62±12.42 years in patients exposed to well water and 69.60±9.58 years in patients not exposed to well water. Exposure to pesticides, farming, rural residence, well water consumption, and pesticides was detected in group 1 (exposed to well water). The standard charts (image of peaks) of data belonging to control were shown in **Appendix 1** to determine that the heavy metal results obtained from ICP-MS are accurate and reliable. The mean duration of well water consumption was 20±6 years (*p*<0.01). Levels of heavy metals and anions, which are risk factors for PD, were significantly higher in well water than in city network water (Table 2; *p*<0.05). Nitrate, sulfate, anion, iron, lithium, and manganese levels were significantly higher in well water than in city network water (*p*<0.05; Tables 2 and 3). Both cases and controls were exposed to ionizing radiation because of proximity to the Metsamor nuclear reactor, located in central Armenia next to Iğdir province. The presence of a positive family history of PD in first-degree relatives revealed that there

was a tendency to PD in both groups. There was no significant difference in cigarette smoking between the 2 groups. Farming, well water consumption, exposure to pesticides, rural birth, and residence in Iğdir province were associated with PD.

**Discussion.** There is substantial evidence indicating that PD in elderly individuals may have its origin in exposure to environmental factors such as well water, pesticides, and ionizing radiation during early life, including young adulthood.<sup>5-9</sup> Our study population stays in Iğdir, an agricultural region of eastern Turkey, where pesticides have been used since 1960. Interestingly, PD prevalence in Iğdir is higher than that in other regions of Turkey. Our results suggest that in Iğdir province, PD prevalence is associated with well water exposure. This study is unique in terms of evaluating the effect of differences in drinking water on long-term health outcomes in Turkey.

Heavy metal and anion levels were higher in well water than in city network water in Iğdir province. Similar to our study, several trials have shown an association between PD and exposure to heavy metals such as thallium, aluminum, bismuth, copper, iron, lead, mercury, cadmium, manganese, and zinc.<sup>10</sup> Although many studies have examined the association between PD and well water consumption, very few studies have

attempted to quantify heavy metal levels and/or specific pesticide contamination in well water.<sup>5-11</sup> Some of these studies found only small relative PD risk, probably due to the absence of heavy metals or other toxins in the consumed well water. Perhaps the wells studied were free of contamination from agricultural chemicals.<sup>12,13</sup>

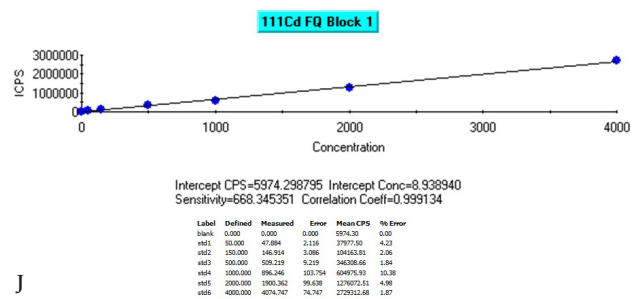
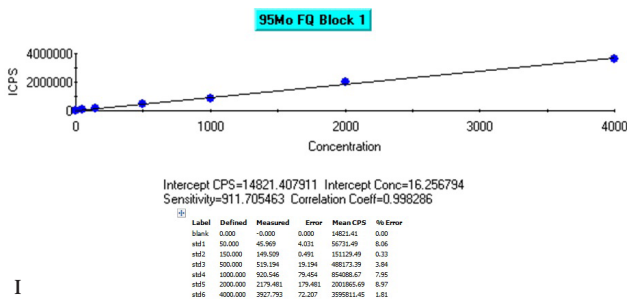
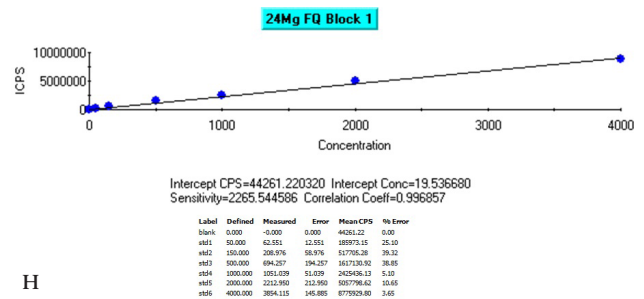
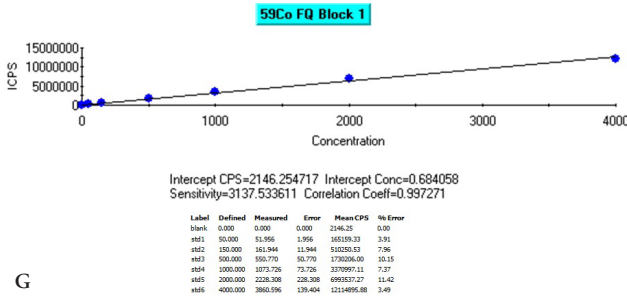
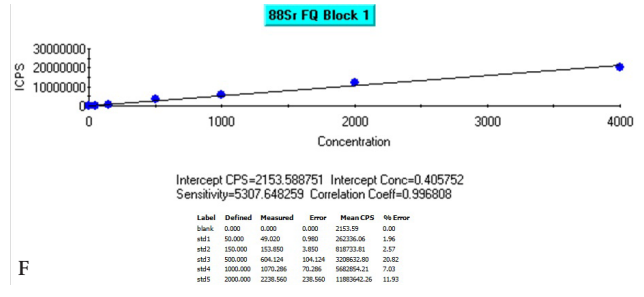
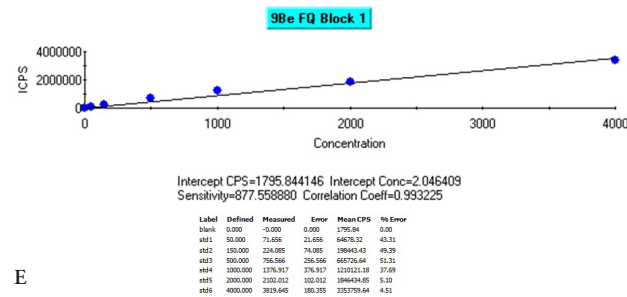
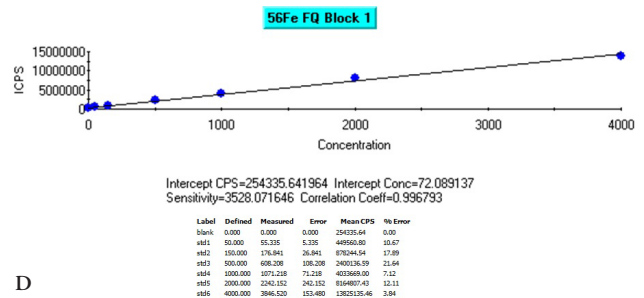
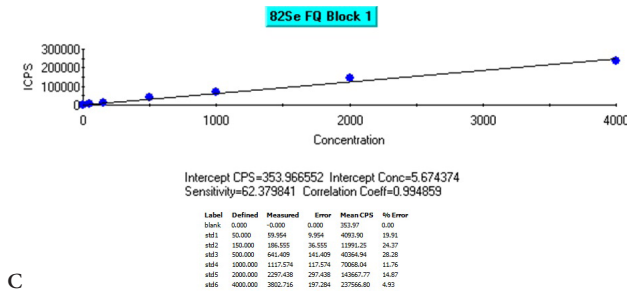
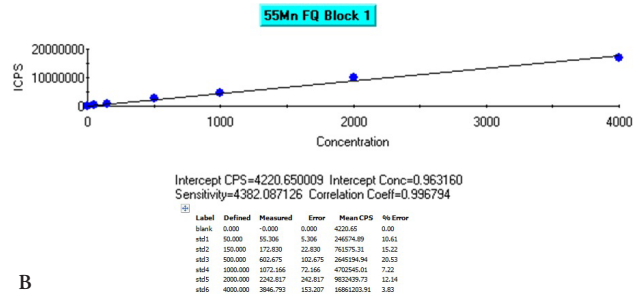
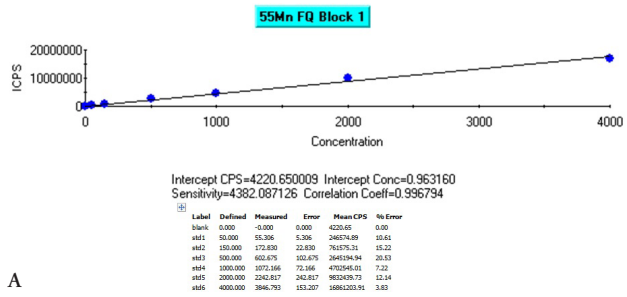
In our study, we also found high sulfate and nitrate levels in well water. In rural areas, particularly among farmers, consumption of well water contaminated with nitrates, phosphates, and heavy metals as a result of chemical fertilization and pesticides is an important risk factor for PD.<sup>13,14</sup> Previous clinical observations have shown that environmental risk factors play a role in PD etiology. Several environmental toxins such as pesticides, herbicides, and nitrates often used in agriculture are risk factors for PD.<sup>14,15</sup> To our knowledge, our study is unique in Turkey because it evaluates the association between PD and well water consumption as well as content. Similar studies should be performed in other farming areas in Turkey to further PD prevention efforts in rural areas.<sup>16</sup>

**Limitation of Study.** Although this study is a prospective controlled study, it has some limitations. The small number of patients and single center are important limitations. However, according to our knowledge, this is the first study to investigate the relationship between heavy metal levels with well water consumption group and city network consumption in Turkey. In order to clarify the details of the relationship between heavy metal levels with well water consumption group and city network consumption, there is needed researches with more multicentre and a higher number of subjects.

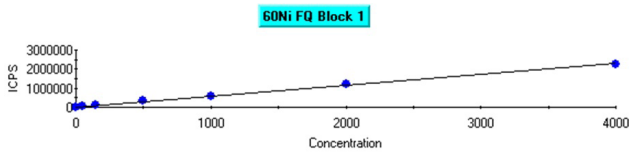
In conclusion, our study is the first in Turkey to evaluate the association between PD and exposure to well water and to evaluate well water content in terms of heavy metals and nitrates. Nitrate, sulfate, iron, lithium, and manganese levels in well water were significantly higher than those in city network water. This study provides argument that consumption of well water in early life take over an eventual role in the etiology of PD in elderly individuals in Iğdir province.

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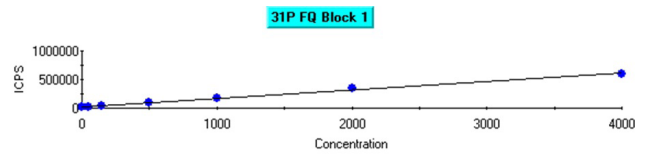
**Appendix 1 -** Standard graphic images that indicate trace element measurements as accurate and reliable. a) Manganese, b) lithium, c) selenium, d) iron, e) beryllium, f) strontium, g) cobalt, h) magnesium, i) molybdenum, j) cadmium



Intercept CPS=656.032145 Intercept Conc=1.151673  
Sensitivity=569.633982 Correlation Coef=0.998617

Label	Defined	Measured	Error	Mean CPS	% Error
blank	0.000	0.000	0.000	656.03	0.00
std1	50.000	57.339	7.339	3338.28	14.68
std2	150.000	176.139	26.139	10223.54	16.76
std3	500.000	596.389	96.389	340279.63	28.28
std4	1000.000	1041.004	41.004	693647.11	4.30
std5	2000.000	2156.960	156.960	1228707.29	7.79
std6	4000.000	3898.623	101.377	2224444.39	2.53

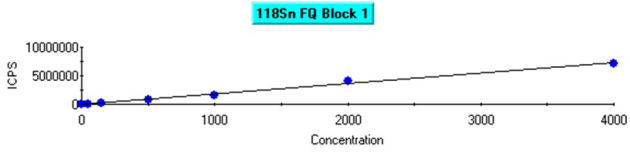
K



Intercept CPS=10028.284481 Intercept Conc=66.755546  
Sensitivity=150.223990 Correlation Coef=0.997260

Label	Defined	Measured	Error	Mean CPS	% Error
blank	0.000	0.000	0.000	10028.28	0.00
std1	50.000	67.862	17.862	20225.84	35.76
std2	150.000	185.189	35.189	37948.15	23.44
std3	500.000	606.091	106.091	101376.15	21.62
std4	1000.000	1071.706	71.706	172024.20	7.17
std5	2000.000	2224.461	224.461	344236.75	11.22
std6	4000.000	3854.778	145.222	589106.47	3.63

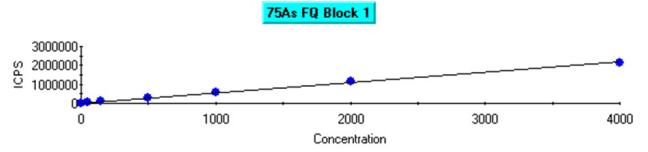
L



Intercept CPS=2570.380628 Intercept Conc=1.409902  
Sensitivity=1823.091354 Correlation Coef=0.996916

Label	Defined	Measured	Error	Mean CPS	% Error
blank	0.000	0.000	0.000	2570.38	0.00
std1	50.000	41.724	6.276	78637.72	16.59
std2	150.000	132.789	17.211	244654.63	11.47
std3	500.000	462.401	37.599	845689.81	7.52
std4	1000.000	812.049	187.950	1482992.76	18.80
std5	2000.000	2207.475	307.475	4020999.32	13.97
std6	4000.000	3948.701	51.299	7051413.43	1.38

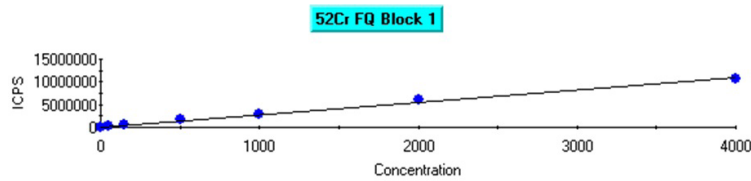
M



Intercept CPS=551.773172 Intercept Conc=1.009660  
Sensitivity=546.494175 Correlation Coef=0.999462

Label	Defined	Measured	Error	Mean CPS	% Error
blank	0.000	-0.000	0.000	551.77	0.00
std1	50.000	53.302	3.302	30773.67	10.60
std2	150.000	159.887	9.887	92301.77	13.26
std3	500.000	577.278	77.278	316030.88	15.46
std4	1000.000	1020.304	20.304	550141.98	2.03
std5	2000.000	2091.713	91.713	1149669.85	4.50
std6	4000.000	3938.956	61.044	2152971.34	1.54

N



Intercept CPS=7988.176039 Intercept Conc=2.921434  
Sensitivity=2734.333817 Correlation Coef=0.997386

Label	Defined	Measured	Error	Mean CPS	% Error
blank	0.000	0.000	0.000	7988.18	0.00
std1	50.000	57.894	7.894	156239.56	15.79
std2	150.000	179.103	29.103	497715.07	19.40
std3	500.000	607.357	107.357	1668706.18	21.47
std4	1000.000	1063.522	63.522	2916011.00	6.35
std5	2000.000	2121.900	217.900	6073487.72	10.50
std6	4000.000	3860.556	139.444	10564037.41	3.49

O

**Appendix 1 -** Standard graphic images that indicate trace element measurements as accurate and reliable. k) nickel, l) phosphorus, m) tin, n) arsenic, o) chrome.