Electronic Supplementary Material (ESI) for Metallomics.

Supplementary Information for R1 of the manuscript by Xu J et al "Evidence from a *post-mortem* study for widespread, substantive brain-copper deficiency in Alzheimer's dementia: emerging target for experimental therapeutic intervention?"

Supplementary Table 1: Tabulated values showing our measurements of the certified NIST standard for each standardised metal in each batch and mean % differences from the certified values

Reference Isotope Date of analysis	23 Na (mmol/kg)	24 Mg (mmol/kg)	39 K (mmol/kg)	44 Ca (mmol/kg)	63 Cu (mg/kg)	66 Zn (mg/kg)	78 Se (mg/kg)
13/02/2015	146.40	0.71	3.59	1.87	1.03	0.74	0.105
18/06/2015	146.68	0.72	3.69	1.77	1.07	0.76	0.114
28/07/2015	142.31	0.71	3.54	1.88	0.98	0.70	0.107
30/07/2015	148.15	0.75	3.75	1.81	1.06	0.77	0.107
14/08/2015	147.88	0.73	3.86	1.95	1.10	0.73	0.105
08/10/2015	135.50	0.70	3.39	1.84	1.05	0.76	0.106
Mean (± SD)	**144.49 (± 4.9)**	**0.719 (± 0.020)**	**3.636 (± 0.166)**	**1.854 (± 0.062)**	**1.047 (± 0.042)**	**0.742 (± 0.026)**	**0.107 (± 0.0030)**
%CV	3.4	2.8	4.6	3.4	4.0	3.5	3.0
NIST certified value	**141.76 (± 0.31)**	**0.696 (± 0.004)**	**3.665 (± 0.025)**	**1.936 (± 0.024)**	**1.008 (± 0.008)**	**0.698 (± 0.030)**	**0.1055 (± 0.0038)**
% difference from NIST certified value	**1.92**	**3.37**	**-0.79**	**-4.25**	**3.85**	**6.26**	**1.78**

Aliquots of plasma (SRM 1950) from the National Institute of Standards and Technology (NIST, Gaithersburg, U.S.A.) were processed with sample batches to provide a standard reference material. NIST SRM 1950 aliquots of 50 µl were digested and processed in the same way as samples. The standard material provides certified reference values for Na, Mg, K, Ca, Cu, Zn and Se in the units given in the table. All measurements across multiple batches were within 5% of the certified values except for zinc, which was 6.26%. The %CV across these batches was less than 5% for all metals.

Supplementary Table 2: Metal concentrations in digestion blanks

	23 Na (µg/L)	24 Mg (µg/L)	39 K (µg/L)	44 Ca (µg/L)	55 Mn (µg/L)	56 Fe (µg/L)	63 Cu (µg/L)	66 Zn (µg/L)	78 Se (µg/L)
Batch 1									
Digestion Blank 1	6.38	0.80	23.5	3.58	0.04	0.02	<0.000	<0.000	<0.000
Digestion Blank 2	10.9	3.11	24.9	11.7	0.04	1.43	<0.000	0.35	<0.000
Lowest Sample	6052	523	8982	190	0.89	201	7.57	42.0	0.66
% highest blank/lowest sample	**0.18**	**0.59**	**0.28**	**6.15**	**4.44**	**0.71**	**<0.000**	**0.84**	**<0.000**
Batch 2									
Digestion Blank 1	22.7	3.13	3.87	11.5	0.03	0.61	0.18	0.25	0.01
Digestion Blank 2	7.36	0.41	5.21	4.04	0.01	0.15	0.03	0.02	0.01
Lowest Sample	4146	441	6552	191	0.91	154	5.91	45.0	0.59
% highest blank/lowest sample	**0.55**	**0.71**	**0.08**	**6.00**	**3.00**	**0.40**	**3.10**	**0.55**	**1.64**
Batch 3									
Digestion Blank 1	11.6	1.91	12.7	4.94	0.02	0.46	0.000157	0.03	0.01
Digestion Blank 2	12.3	2.23	6.67	3.66	0.01	0.52	0.02	0.12	0.01
Lowest Sample	6348	497	8631	207	0.99	118	5.33	38.4	0.55
% highest blank/lowest sample	**0.19**	**0.45**	**0.15**	**2.39**	**2.26**	**0.44**	**0.44**	**0.31**	**2.68**
Blank Batch (n=25)									
Mean (± SD)	1.38 (± 3.97)	3.43 (± 0.68)	2.71 (± 7.77)	5.76 (± 3.16)	0.02 (± 0.01)	0.61 (± 0.36)	0.05 (± 0.03)	0.16 (± 0.10)	0.004 (± 0.0039)
Highest blank	18.98	5.12	26.93	12.44	0.06	1.82	0.15	0.35	0.02
Lowest sample	4146	441	6552	190	0.89	118	5.33	38.4	0.55
% Highest blank/lowest sample	**0.458**	**1.16**	**0.411**	**6.55**	**6.93**	**1.56**	**2.73**	**0.905**	**2.99**

<0.000 indicates that a sample concentration was lower than that of the calibration blank. Samples were analysed across three batches. Each sample batch included two digestion blanks, and tubes containing standard-containing acid but no sample to ensure background levels from processing were not high enough to interfere with sample quantification. A blank batch consisting of 25 digestion blanks was analysed in order to assess the variability of metal levels across individual tubes as also used for tissue digestion. Whilst some variability between different tubes was observed, for most metals the highest blank was still less than 5% of the lowest sample measured across all of the sample batches. The exceptions were calcium and manganese where the highest blanks were 6.55% and 6.93% of the lowest samples respectively; these values are nevertheless indicative of excellent analytical capability of these methods. For sodium, magnesium, potassium and calcium the highest blanks occurred in samples run immediately after QC samples so are likely higher due to carryover rather than higher background levels in the corresponding tubes.

Supplementary Table 3: Detection limit, limit of quantitation and background equivalent concentration for each physiological metal measured in this study

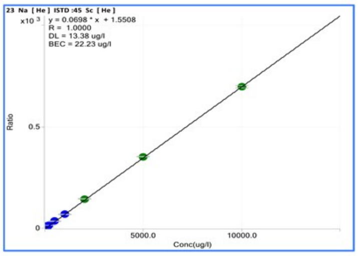
		23 Na (µg/L)	24 Mg (µg/L)	39 K (µg/L)	44 Ca (µg/L)	55 Mn (µg/L)	56 Fe (µg/L)	63 Cu (µg/L)	66 Zn (µg/L)	78 Se (µg/L)
Lowest Standard		**50**	**50**	**50**	**50**	**0.5**	**50**	**0.5**	**0.5**	**0.5**
Batch 1	DL	13.4	0.622	2.84	7.35	0.0222	0.345	0.259	0.0905	0.0359
	LOQ	100	50	200	100	0.5	50	2	1	0.5
	BEC	22.3	3.39	58.0	9.56	0.0196	0.587	0.487	0.266	0.0233
	Lowest Sample	**6052**	**523**	**8982**	**190**	**0.89**	**201**	**7.57**	**42.0**	**0.66**
Batch 2	DL	0.937	0.546	6.04	10.6	0.00567	0.159	0.0214	0.0636	0.00690
	LOQ	50	50	200	100	0.5	50	0.5	2	0.5
	BEC	18.3	2.57	58.4	5.62	0.0132	0.202	0.0512	0.114	0.0139
	Lowest Sample	**4146**	**441**	**6552**	**191**	**0.91**	**154**	**5.91**	**45.0**	**0.59**
Batch 3	DL	2.20	0.295	4.17	8.11	0.0171	0.274	0.00392	0.0778	0.0181
	LOQ	100	50	200	100	0.5	50	0.5	1	0.5
	BEC	15.7	2.88	59.9	6.02	0.0216	0.063	0.0852	0.168	0.0116
	Lowest Sample	**6348**	**497**	**8631**	**207**	**0.99**	**118**	**5.33**	**38.4**	**0.55**

***Nonstandard abbreviations: BEC-Background equivalent concentration; DL-Detection limit.** Lowest calibration standards analysed were 50µg/L for Na, Mg, K, Ca and Fe and 0.5µg/L for all other metals. For each metal, all samples analysed were higher than the lowest standard. The software employed (Mass Hunter, Agilent) automatically calculated values for DLs (detection limits) and BECs (background equivalent concentrations) corresponding to each element analysed. LOQs (limits of quantitation) were calculated by comparison of calibration blanks and standards. Value shown for lowest sample are lowest raw measurements without correction for corresponding tissue mass.

Supplementary Figure 1: Standards curves for each physiological metal measured in this study.

Batch 1

Na



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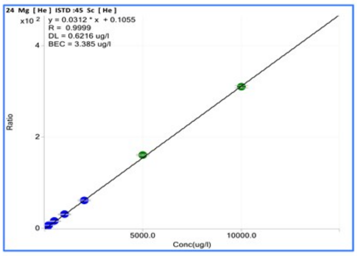

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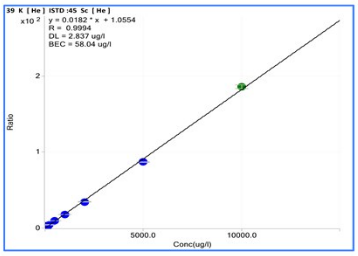

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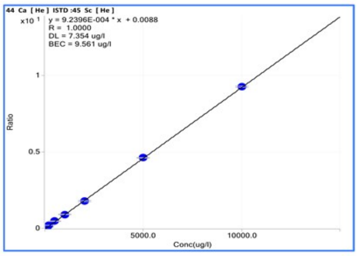

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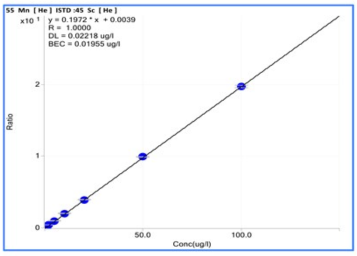

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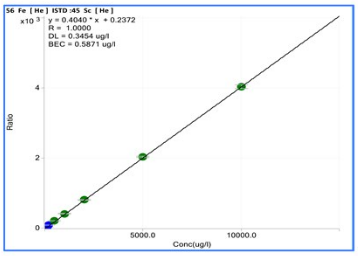

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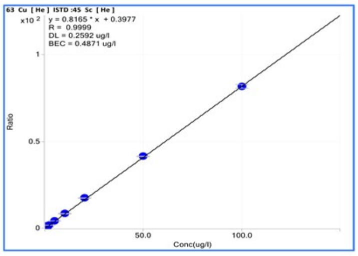

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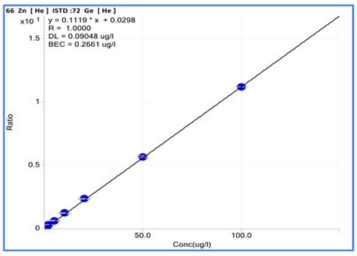

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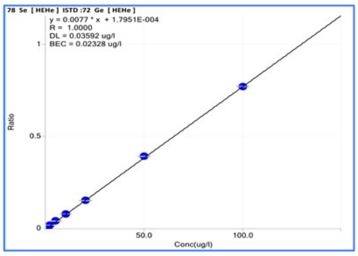

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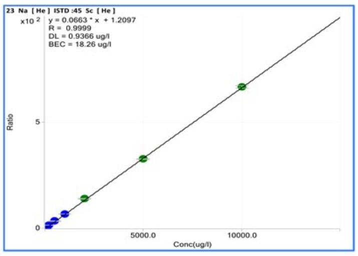
Na



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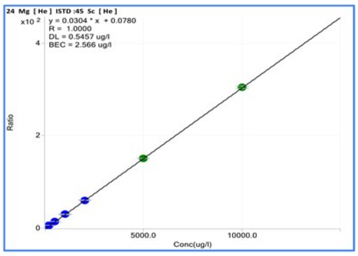

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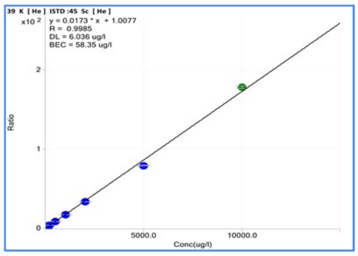

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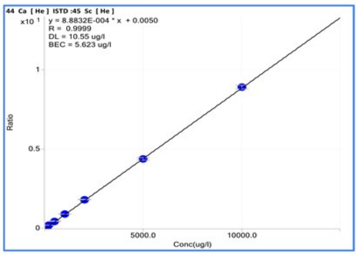

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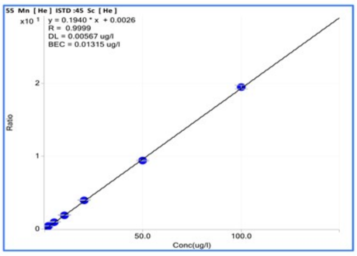

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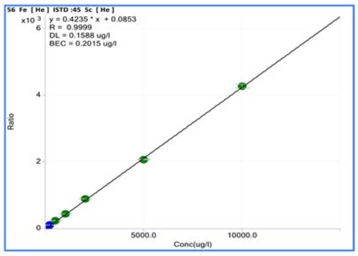

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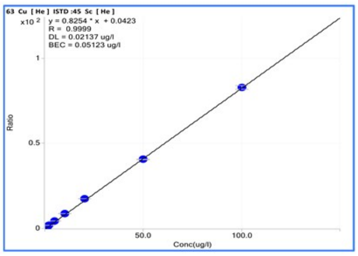

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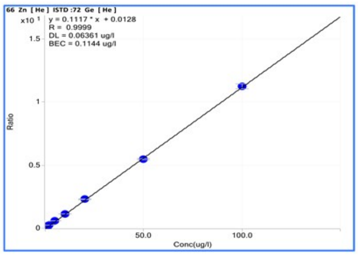

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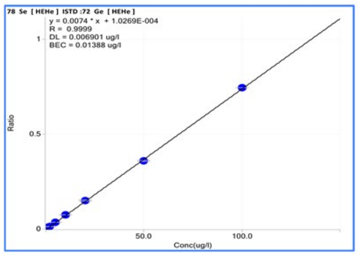

Batch 3

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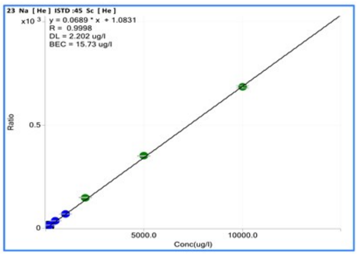

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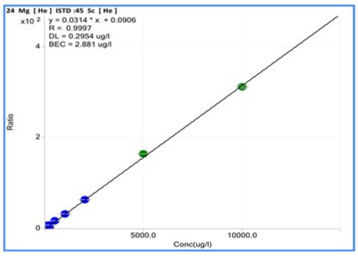

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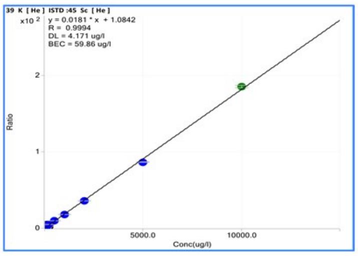

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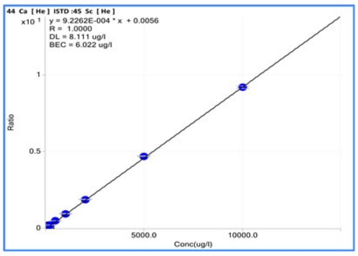

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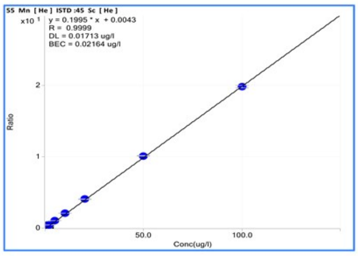

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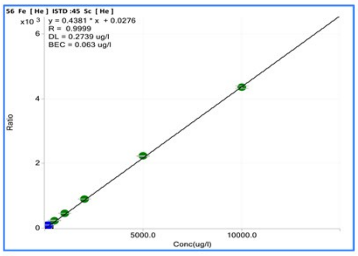

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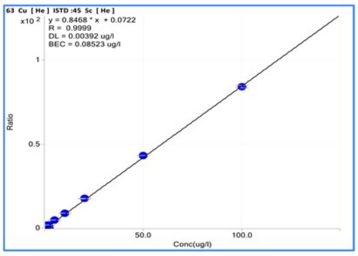

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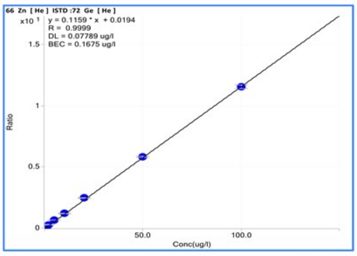

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