Application Note
Foods

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Determination of Heavy Metals and Trace Elements in Alternative Meats Per EAM 4.7 Method for ICP-MS

Food safety analysis of plant-based protein foods and cell cultures using ICP-MS

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Introduction

People need to consume sufficient amounts of essential macronutrients—carbohydrates, fats, and protein—to support their body's energy needs. Protein is needed for growth, development, and repair of body tissues, and is especially important for building or maintaining muscles and for bone-health. Meat, poultry, fish, dairy products, and eggs are a major source of protein while plant-based sources include soya, beans, nuts, lentils, grains, cereals, fruit, and vegetables. Whether for ethical, dietary, health, or social reasons, there are increasing numbers of people globally who follow a vegan or vegetarian diet, or who are reducing their intake of animal-based foods. Concern about the impact of intensively farmed animals on the climate and natural resources may persuade even more people to limit the amount of meat in their diets (*1*).

The food industry is aware of the rise in popularity of meat-free foods and is actively investigating processes and products that can help meet the demand. The trend for meat-free foods can be seen by the ever-increasing selection of alternative protein products on supermarket shelves, and on the menus of fast-food outlets and restaurants. Some food companies are already selling products that are produced by cultivating meat-tissue from animal-origin cells grown in a bioreactor.

To ensure that non-meat based protein or cultured protein products are safe for human consumption, manufacturers must comply with Good Manufacturing Practice (GMP). Typically, GMP guidelines provide guidance for manufacturing, testing, and quality assurance of foods. Food safety analysis includes testing for chemicals, e.g., organic contaminants such as pesticide residues, and inorganic contaminants such as heavy metals, which are controlled in foodstuffs. In the United States (US), the Food and Drug Administration (FDA) regulates a wide range of foods and publishes analytical methods that laboratories should use to help ensure food safety. For example, FDA Elemental Analysis Manual (EAM) 4.7 is a comprehensive method that describes how to determine 12 elements in food digests (prepared using microwave assisted acid decomposition) by ICP-MS. EAM 4.7 also outlines a series of quality control (QC) tests to ensure that analysts can demonstrate instrument performance and data accuracy (*2*). Companies wanting to produce, import, or export cell-based alternative meats may need regulatory approval in each target market. However, it is likely that existing analytical testing of foods, such as EAM 4.7, can be applied to any newly developed cell-cultivated food products.

This study describes the use of the Agilent 7850 ICP-MS and Agilent SPS 4 autosampler for the analysis of 30 elements in different plant-based alternative meat samples and 29 elements in cell-culture solutions. Be was included in the plant-based protein analysis suite but was not an analyte of interest in the cell culture media study. The analytical method was adapted from a previous foods analysis study using the 7850 ICP-MS (*3*). The list of elements included all 12 heavy metal and trace elements specified in EAM 4.7: arsenic, cadmium, chromium, copper, lead, manganese, mercury, molybdenum, nickel, selenium, thallium, and zinc. In addition, the following trace and major elements were analyzed: aluminum, antimony, barium, beryllium, boron, calcium, cobalt, iron, magnesium, phosphorus, potassium, silver, sodium, strontium, sulfur, tin, titanium, and vanadium.

The quality of the data obtained for the elements analyzed was assessed through the measurement of four food certified reference materials (CRMs), a fortified method blank (FMB), and four fortified analytical portions (FAPs) of plant-based meat alternative foods. FAP refers to samples that are spiked before sample preparation. An FMB of the liquid cell media and FAPs of cell media, spent cell media, and conditioned spent cell media were also prepared and analyzed in this study.

Experimental

Calibration standards

The calibration standards were prepared in 2% nitric acid (HNO_3) and 0.5% hydrochloric acid (HCl). HCl is routinely added to samples for analysis using Agilent ICP-MS systems, as it ensures that chemically unstable elements such as Hg are retained in solution. Any Cl-based polyatomic overlaps formed are easily controlled using the standard helium (He) collision cell mode (*4*). Calibration standards were prepared from Agilent standard solutions including environmental calibration standard, p/n 5183-4688. Agilent single calibration standards were used for Hg (p/n 5190-8485), S (p/n 5190-8210), P (p/n 5190-8428, B (p/n 5190-8254), Ti (p/n 5190-8545), Sr (p/n 5190-8527), and Sn (p/n 5190-8543). Most trace elements were calibrated from 0.1 to 25 ppb. Cu, Zn, and Mn were calibrated up to 250 ppb. Hg was calibrated from 0.01 to 2.5 ppb. Mineral elements were calibrated from 5 to 25,000 ppb.

The internal standard (ISTD) solution containing 2 ppm ^{6}Li, Sc, Ge, Rh, Tb, and Bi (Agilent p/n 5188-6525) was prepared in 1% HNO_3, 0.5% HCl, and 10% isopropanol (IPA). Per the 4.7 method, IPA was added to the ISTD to ensure a consistent level of carbon in the solutions analyzed. This approach helps avoid the ionization enhancement that can affect As and Se sensitivities when variable levels of residual carbon are present in the samples after microwave digestion. The ISTD solution was added automatically online at a flow rate approximately 16 times lower than the sample flow.

Reference materials and samples

Four food matrix SRMs from National Institute of Standards and Technology (NIST, Gaithersburg, US) were used to validate the method. The SRMs included NIST 1577c Bovine Liver, NIST 1947 Lake Michigan Fish Tissue, NIST 1549a Whole Milk Powder, and NIST 1568b Rice Flour. The plant-based alternative meat samples (non-meat equivalents of fried chicken, beef burger, and minced beef) were bought in a supermarket in North Carolina, USA. A range of culture media liquid samples were obtained from a research project performed at UC Davis (*5*). The formulation of the cell culture media consisted of 40% Dulbecco's Modified Eagle Medium (DMEM), 40% Ham's F-10 Nutrient Mixture, 20% fetal bovine serum (FBS).

Standard and sample preparation

All the SRMs and plant-based alternative meat food samples were prepared as received (without homogenization or moisture removal) according to the digestion procedure outlined in the EAM 4.7 method. A MARS 6 closed-vessel microwave digestion system from CEM Corporation, USA was used. After accurately weighing the samples (approximately 0.5 g of food or SRM) into 75 mL PFA Xpress vessels, 8 mL of HNO_3 and 1 mL of H_2O_2 were added to the vessels. Duplicates of the samples, SRMs, and spiked samples (FAPs) were then digested in a single batch, using the heating program shown in Table 1. Each digestion batch can accommodate up to 40 varied food sample matrices, with a single program being used for all sample types. Following digestion, 0.5 mL concentrated HCl was added to the digests, followed by de-ionized water to a final weight of 100 g.

Table 1. Microwave digestion parameters.

Parameter	Setting
Power (W)	1800
Ramp Time (min)	25
Hold Time (min)	15
Temperature (°C)	200

Eight samples of the liquid cell culture media formulated for the cultivation of alternative meat samples at UC Davis were also analyzed in this study.

Samples 1 and 2 were from different batches of the same unused cell media formulation that had been incubated for different amounts of time. As a result, a slight difference in chemical composition was expected. Sample 1 was fresh media from the bottle, while sample 2 was analyzed after having been kept in the incubator for 21 days.

Samples 3, 4, and 5 were spent cell-culture media from the same batch, collected after the media had been used to grow primary embryonic chicken muscle precursor cells for 21 days. Spent media is the cell culture media that remains after the cells have been harvested and so contains unused nutrients and accumulated metabolites and waste products. Samples 3 to 5 were expected to be similar in composition, since they were "biological replicates", i.e. media used to incubate three separate cultures of the same type of cells. As a result, any differences in chemical composition between the three samples should be attributable to metabolic variation between the replicate cultures.

Samples 6, 7, and 8 were samples of the same batch of media used for samples 3, 4, and 5, but sampled after the cells had been growing in the media for 14, rather than 21, days. Samples 6, 7, and 8 were also biological replicates, used to grow three separate cultures of the same type of chicken embryo cells as were used for samples 3 to 5.

A 15 mg aliquot of each of the liquid cell culture media samples was diluted (rather than digested) in 15 mL of 2% HNO_3 and 0.5% HCl, before being analyzed directly by ICP-MS. Cell culture media is valuable, so the sample size was limited to 15 mg.

The analytical sequence of calibration standards, samples, and QC solutions is shown in Figure 1. The sample block was analyzed repeatedly with automatic insertion of the periodic QC block after every 10 samples.

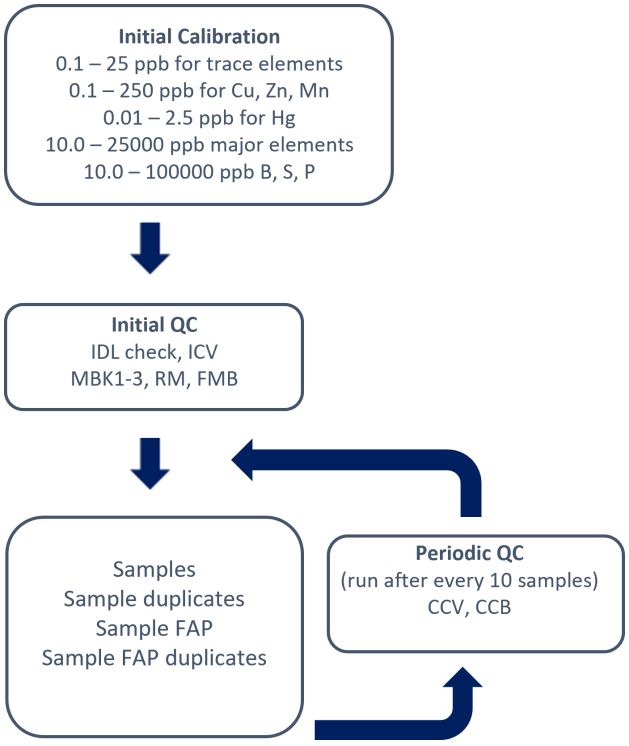

Figure 1. Analytical sequence.

Key: Instrument detection limit (IDL), initial calibration verification (ICV), method blank (MBK), reference material (RM), fortified method blank (FMB), fortified analytical portion (FAP), continuing calibration verification (CCV), continuing calibration blank (CCB).

Instrumentation

An Agilent 7850 ICP-MS, which includes the ORS^4 collision cell and UHMI aerosol dilution system, was used for the analysis. The standard ICP-MS sample introduction system was used, consisting of a MicroMist glass concentric nebulizer, temperature-controlled quartz spray chamber, and quartz torch with 2.5 mm id injector. A nickel-plated copper sampling cone was used, together with a nickel skimmer cone.

Based on previous experience of testing food digests (*3*), the preset plasma mode HMI-4 was selected, which applies an aerosol dilution factor of four times to the samples (*6*). When UHMI is selected, plasma settings are autotuned as appropriate for the matrix levels of the target sample types, as indicated by the shaded rows in Table 2. Other instrument operating conditions were optimized automatically using the ICP-MS MassHunter autotune function. All analytes were acquired in helium (He) mode (enhanced He mode for P, S, As, and Se). EAM 4.7 stipulates that an ICP-MS used for FDA regulated food analysis must be able to operate in helium mode with kinetic energy discrimination (KED). Reactive cell gases are not an acceptable alternative on single quadrupole ICP-MS, due to the risk of creating new spectral overlaps through the formation of reaction product ions. Operating the ORS^4 in He mode is the standard method used on Agilent ICP-MS systems, as it can reliably remove the typical polyatomic ion interferences on all common analytes (*4, 7*). Instrument operating conditions are listed in Table 2.

Table 2. Agilent 7850 ICP-MS operating conditions*.

ICP-MS Parameter	Setting
RF Power (W)	1600
Sampling Depth (mm)	10
Carrier Gas Flow (L/min)	0.80
Dilution (UHMI) Gas Flow (L/min)	0.15
Lens Tune	Autotune
Helium Cell Gas Flow (mL/min)	4.3 (10**)
Energy Discrimination (V)	5 (7**)

** Shaded parameters are defined in the method and HMI-4 plasma presets; all parameters were automatically optimized during start-up and autotuning. ** Enhanced He mode settings used for P, S, As, and Se.*

Results and discussion

Representative calibration curves are presented in Figure 2. The plots for Na, Mg, Mn, Cu, As, and Hg show excellent linearity across the calibrated range, with correlation coefficients of 0.9999 or better.

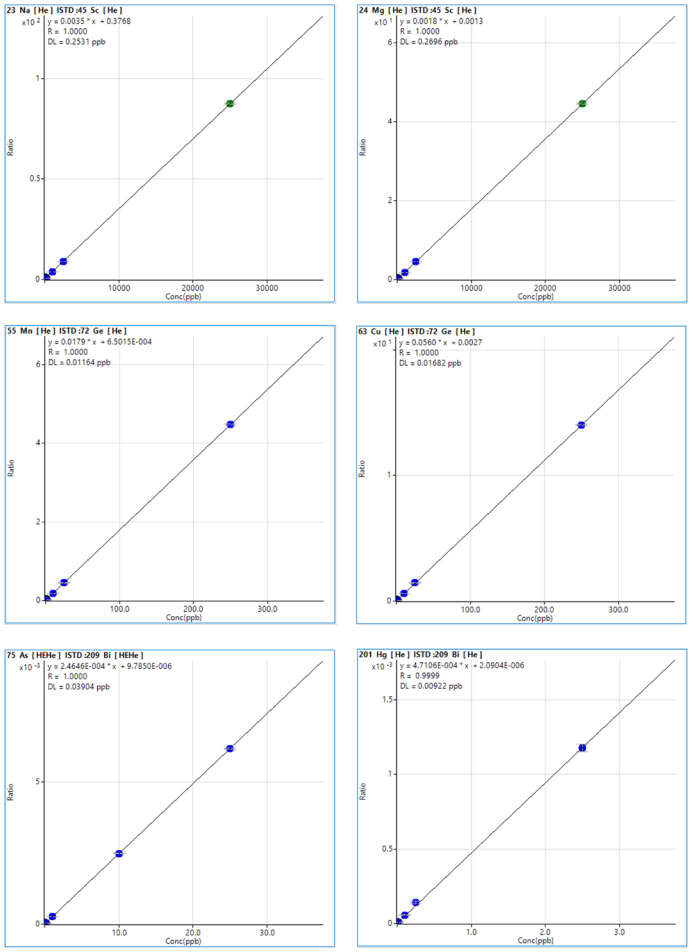

Figure 2. Representative calibration curves for major and trace elements.

Typical 7850 ICP-MS instrument detection limits (DLs) calculated from the ICP-MS MassHunter calibrations are shown in Table 3. The EAM method detection (LOD) and quantification limits (LOQ) – also shown in Table 3 – were calculated based on method blanks measured at the end of the run, n=10 (*8*). Data was acquired for 30 elements, including the 12 elements required by EAM 4.7, using He cell gas for all analytes.

Table 3. Agilent 7850 ICP-MS detection limits and EAM 4.7 nominal analytical limits, where provided.

Element	ICP-MS MassHunter		Calculated Based on EAM 4.7 Analytical Limits		EAM 4.7 Nominal Analytical Limits	
	DL (µg/kg)	BEC (µg/kg)	LOD (µg/kg)	LOQ (µg/kg)	LOD (µg/kg)	LOQ (µg/kg)
9 Be	0.000	0.000	0.011	0.037	-	-
11 B	4.290	8.808	1.501	5.002	-	-
23 Na	7.410	275.1	7.505	25.02	-	-
24 Mg	0.140	0.384	0.141	0.471	-	-
27 Al	0.100	0.423	0.204	0.680	-	-
31 P	1.650	3.475	2.372	7.908	-	-
34 S	242.0	911.3	212.9	709.8	-	-
39 K	13.58	152.0	4.311	14.37	-	-
43 Ca	6.450	8.585	5.955	19.85	-	-
47 Ti	0.219	0.110	0.289	0.962	-	-
51 V	0.012	0.060	0.015	0.049	-	-
52 Cr	0.035	0.433	0.032	0.107	5.390	48.90
55 Mn	0.021	0.032	0.010	0.033	2.330	21.20
56 Fe	0.005	0.787	0.053	0.175	-	-
59 Co	0.001	0.002	0.001	0.003	-	-
60 Ni	0.024	0.024	0.006	0.020	6.380	58.00
63 Cu	0.006	0.055	0.018	0.060	6.020	54.70
66 Zn	0.159	1.003	0.116	0.387	37.40	340.0
75 As	0.029	0.043	0.004	0.014	1.270	11.60
78 Se	0.166	0.412	0.088	0.292	7.280	66.10
88 Sr	0.004	0.008	0.002	0.006	-	-
95 Mo	0.005	0.002	0.003	0.012	5.180	47.10
107 Ag	0.001	0.002	0.002	0.005	-	-
111 Cd	0.003	0.003	0.003	0.010	0.408	3.710
118 Sn	0.011	0.129	0.008	0.025	-	-
121 Sb	0.013	0.033	0.007	0.024	-	-
137 Ba	0.017	0.008	0.017	0.058	-	-
201 Hg	0.006	0.006	0.012	0.039	0.861	7.820
205 Tl	0.001	0.004	0.013	0.044	*0.281	*2.100
Pb**	0.002	0.024	0.001	0.005	1.200	10.90

*All elements were acquired in He mode (enhanced He for P, S, As, and Se). The Nominal Analytical Limits are given in EAM 4.7 and are based on method blanks measured during the single lab validation over one year; n = 143. *Based on a single lab validation (n = 27). **Pb was measured as the sum of the three most abundant isotopes, 206, 207, and 208.*

Verification of instrument calibration and sample digestion process

As part of the method quality control procedure specified in EAM 4.7, and to ensure the ongoing validity of the calibration, a CCV standard was analyzed five times during the analytical sequence. Most tested elements reported recoveries within the EAM acceptance criteria of ±10% of the actual concentration of the CCV (results not shown).

To verify the sample digestion process and the accuracy of the analytical method, two sets of the four NIST SRMs were analyzed in duplicate using the 7850 ICP-MS. As shown in Table 4, the mean concentrations were in good agreement with the certified concentrations, meeting the QC criteria requirements of the FDA EAM method of 80–120%. Since not all SRMs are certified for all analytes, blank cells indicate the absence of a certified or reference value.

Table 4. Mean measured concentrations of four NIST food-based SRMs using the Agilent 7850 ICP-MS. Mean calculated from triplicate sample digestion, each run in triplicate, n=9.

Element	NIST 1577c Bovine Liver					NIST 1947 Lake Michigan Fish Tissue				
	Conc Unit	Certified Conc	Mean Measured Conc	Recovery (%)*	QC Criteria (80–120%)**	Conc Unit	Certified Conc	Mean Measured Conc	Recovery (%)*	QC Criteria (80–120%)**
^{23}Na	mg/kg	2033	2039	100	Pass	-	-	-	-	-
^{24}Mg	mg/kg	620	614	99	Pass	-	-	-	-	-
^{31}P	mg/kg	11,750 R	12,189	104	Pass	-	-	-	-	-
^{34}S	mg/kg	7490	7541	101	Pass	-	-	-	-	-
^{39}K	mg/kg	10,230	10,195	100	Pass	-	-	-	-	-
^{43}Ca	mg/kg	131	115	88	Pass	-	-	-	-	-
^{51}V	µg/kg	8.17	8.52	104	Pass	-	-	-	-	-
^{52}Cr	µg/kg	53	57	107	Pass	-	-	-	-	-
^{55}Mn	mg/kg	10.46	10.18	97	Pass	mg/kg	0.076	0.071	93	Pass
^{56}Fe	mg/kg	197.94	199.86	101	Pass	mg/kg	3.79	3.38	89	Pass
^{59}Co	mg/kg	0.300	0.307	102	Pass	-	-	-	-	-
^{60}Ni	µg/kg	44.5	49.3	111	Pass	-	-	-	-	-
^{63}Cu	mg/kg	275.2	256.8	93	Pass	mg/kg	0.411	0.356	87	Pass
^{66}Zn	mg/kg	181.1	181.7	100	Pass	mg/kg	2.66	2.44	92	Pass
^{75}As	µg/kg	19.6	22.7	116	Pass	mg/kg	0.732	0.672	92	Pass
^{78}Se	mg/kg	2.031	2.182	107	Pass	mg/kg	0.475	0.426	90	Pass
^{88}Sr	µg/kg	95.3	96.8	102	Pass	-	-	-	-	-
^{95}Mo	mg/kg	3.30	3.49	106	Pass	-	-	-	-	-
^{107}Ag	µg/kg	5.9	6.1	104	Pass	-	-	-	-	-
^{111}Cd	µg/kg	97.0	98.4	101	Pass	-	-	-	-	-
^{121}Sb	µg/kg	3.13 R	3.74	120	Pass	-	-	-	-	-
^{201}Hg	µg/kg	5.36 R	5.93	111	Pass	mg/kg	0.254	0.274	108	Pass
Pb	µg/kg	62.8	63.6	101	Pass	-	-	-	-	-

Pb was measured as the sum of the three most abundant isotopes, 206, 207, and 208.

Table 4 continues on next page

Table 4 continued...

Element	NIST Whole Milk Powder SRM 1549a					NIST Rice Flour SRM 1568b				
	Conc Unit	Certified Conc	Mean Measured Conc	Recovery (%)*	QC Criteria (80–120%)**	Conc Unit	Certified Conc	Mean Measured Conc	Recovery (%)*	QC Criteria (80–120%)**
^{23}Na	mg/kg	3176	3648	115	Pass	-	-	-	-	-
^{24}Mg	mg/kg	892	1018	114	Pass	mg/kg	559	525	94	Pass
^{31}P	mg/kg	7600	8792	116	Pass	mg/kg	1530	1711	112	Pass
^{39}K	mg/kg	11920	13673	115	Pass	mg/kg	1282	1307	102	Pass
^{43}Ca	mg/kg	8810	10195	115	Pass	mg/kg	118.4	125.0	105	Pass
^{52}Cr	-	-	-	-	-	mg/kg	118.4	124.5	105	Pass
^{55}Mn	mg/kg	0.184	0.189	103	Pass	-	19.2	19.2	100	Pass
^{56}Fe	mg/kg	1.85 R	2.12	115	Pass	-	7.42	7.68	104	Pass
^{63}Cu	-	-	-	-	-	mg/kg	2.35	2.39	102	Pass
^{66}Zn	mg/kg	33.8	34.7	103	Pass	mg/kg	19.42	18.55	96	Pass
^{75}As	-	-	-	-	-	mg/kg	0.285	0.335	118	Pass
^{78}Se	mg/kg	0.242	0.288	119	Pass	mg/kg	0.365	0.425	116	Pass
^{111}Cd	-	-	-	-	-	mg/kg	0.0224	0.0201	90	Pass
^{201}Hg	-	-	-	-	-	µg/kg	5.91	6.00	107	Pass

*R - Reference mass fraction values. * FDA Elemental Analysis Manual (Section 3.4 Special Calculations) 3.4 Equation 20. ** FDA EAM 4.7 QC Criteria (80–120%) for NIST certified values.*

Matrix effects and spike recoveries

To test for nonspectral interferences (matrix effects), an FMB was prepared by spiking the blank at 1 µg/kg for most trace elements, 50 µg/kg for Al, Fe, Cu, Zn, and 4000 µg/kg for major elements including K, P, and S. The FMB was analyzed periodically throughout the entire analysis run. All recoveries were within the EAM 4.7 method acceptable % recovery range of 90–110%, as shown in Table 5.

A spike recovery (FAP) test was carried out to check the accuracy of the 7850 ICP-MS method for the analysis of the plant-based (meat-substitute) food products. Each sample was spiked with all elements at 1 or 50 µg/kg and measured using the 7850 ICP-MS. For samples that had naturally occurring elemental concentrations below 1 µg/kg, a 1 µg/kg spike is reported. For samples with higher naturally occurring concentrations, the 50 or 4000 µg/kg spike results are reported. The recoveries for all elements in the fortified plant-based beef-substitute food samples were within the EAM 4.7 method QC criteria of ±20%, as shown in Table 5.

Table 5. The mean recovery results are based on the analysis of replicate sample digests, each run in duplicate on the Agilent 7850 ICP-MS, n=2. The low spike concentration was 1 µg/kg and the high spike concentration was 50 or 4000 µg/kg.

		Method Blank			Plant-based "Minced Beef"		
	Conc Unit	Method Blank Conc	Recovery Low Spike (%)	Recovery High Spike (%)	Native Conc	Recovery Low Spike (%)	Recovery High Spike (%)
11 B	µg/kg	7.472	-	93	<LOD	-	111
23 Na	mg/kg	15.98	-	108	3650	-	*
24 Mg	mg/kg	<LOD	-	105	210.5	-	*
27 Al	µg/kg	0.448	106	101	2659	-	103
31 P	mg/kg	<LOD	-	96	2116	-	*
34 S	mg/kg	<LOD	-	**	1371	-	*
39 K	mg/kg	<LOD	-	105	2655	-	*
43 Ca	mg/kg	<LOD	-	102	1565	-	*
47 Ti	µg/kg	<LOD	102	91	152	-	103
51 V	µg/kg	<LOD	106	-	15.3	-	104
52 Cr	µg/kg	<LOD	106	-	89.2	-	103
55 Mn	µg/kg	<LOD	-	98	4085	-	104
56 Fe	mg/kg	0.859	-	106	33.16	-	*
59 Co	µg/kg	<LOD	105	-	35.2	-	104
60 Ni	µg/kg	<LOD	106	-	188	-	103
63 Cu	µg/kg	0.053	-	107	1615	-	105
66 Zn	mg/kg	<LOD	-	103	44.54	-	*
75 As	µg/kg	<LOD	104	-	16.3	-	109
78 Se	µg/kg	<LOD	104	-	78.0	101	98
88 Sr	µg/kg	<LOD	95	-	1686	-	92
95 Mo	µg/kg	<LOD	105	-	275	-	105
107 Ag	µg/kg	<LOD	-	102	1.481	-	92
111 Cd	µg/kg	<LOD	106	-	9.470	-	104
118 Sn	µg/kg	3.580	91	-	634	NA	
121 Sb	µg/kg	<LOD	104	-	<LOD	96	-
137 Ba	µg/kg	<LOD	103	-	391	-	100
201 Hg	µg/kg	<LOD	101	-	<LOD	111	-
205 Tl	µg/kg	<LOD	98	-	9.341	110	-
Pb***	µg/kg	<LOD	104	-	6.697	114	-

*All elements were acquired in He mode (enhanced He for P, S, As, and Se). *Spike level too low compared to native concentration, ** below calibration range. NA = not applicable, as not measured. ***Pb was measured as the sum of the three most abundant isotopes, 206, 207, and 208.*

Quantitative results for plant-based protein food samples

Quantitative results are given in Table 6 for three plant-based, alternative meat food samples. In addition to the 12 elements specified in EAM 4.7, data is provided for Be, B, Na, Mg, Al, P, S, K, Ca, Ti, V, Fe, Co, Sr, Ag, Sn, Sb, and Ba.

Table 6. Quantitative results (n=9) measured using the Agilent 7850 ICP-MS for three plant-based meat-alternative food samples.

	Conc Unit	Plant-based Fried "Fried Chicken"	Plant-based "Beef Burger"	Plant-based "Minced Beef"
9 Be	µg/kg	3.29 ± 2.64	3.51 ± 2.75	<LOD
11 B	µg/kg	2952 ± 204.6	2753 ± 153.5	<LOD
23 Na	mg/kg	10293 ± 350	2537 ± 48	3650 ± 231
24 Mg	mg/kg	281 ± 26360	627 ± 24679	210 ± 34
27 Al	µg/kg	65704 ± 28678	1291 ± 124.9	2659 ± 520.7
31 P	mg/kg	3291 ± 242784	1874 ± 76	2116 ± 312
34 S	mg/kg	24576 ± 434	1756 ± 68	1371 ± 237
39 K	mg/kg	1920± 91	4416 ± 86	2655 ± 161
43 Ca	mg/kg	169 ± 9	1025 ± 38	156 ± 22
47 Ti	µg/kg	63362 ± 17691	81.80 ± 11.98	152.4 ± 44.53
51 V	µg/kg	42.92 ± 11.86	8.21 ± 2.92	15.35 ± 1.43
52 Cr	µg/kg	190.7 ± 58.17	178.9 ± 8.71	89.19 ± 13.72
55 Mn	µg/kg	5610 ± 592.5	11450 ± 561.5	4085 ± 856.3
56 Fe	µg/kg	43416 ± 5195	33040 ± 410.0	33159 ± 6752
59 Co	µg/kg	583.5 ± 142.3	1085 ± 75.93	35.17 ± 4.59
60 Ni	µg/kg	581.8 ± 25.01	147.2 ± 19.20	188.5 ± 12.62
63 Cu	µg/kg	4201 ± 861.6	2295 ± 42.85	1615 ± 286.5
66 Zn	µg/kg	13990 ± 989.2	46159 ± 405.1	44540 ± 3098
75 As	µg/kg	30.39 ± 4.48	12.97 ± 3.21	16.35 ± 2.40
78 Se	µg/kg	82.22 ± 28.25	67.56 ± 11.85	78.04 ± 11.96
88 Sr	µg/kg	1272 ± 160.2	2467 ± 157.0	1686 ± 165.5
95 Mo	µg/kg	902.2 ± 121.9	856.1 ± 22.26	274.8 ± 45.87
107 Ag	µg/kg	14.10 ± 18.89	2.41 ± 0.76	1.48 ± 0.41
111 Cd	µg/kg	14.07 ± 1.34	9.91 ± 1.54	9.47 ± 1.28
118 Sn	µg/kg	684.7 ± 24.55	697.8 ± 26.94	634.2 ± 13.62
121 Sb	µg/kg	<LOD	<LOD	<LOD
137 Ba	µg/kg	861.2 ± 44.50	2691 ± 177.15	390.6 ± 80.28
201 Hg	µg/kg	<LOD	<LOD	<LOD
205 Tl	µg/kg	34.15 ± 29.97	7.15 ± 1.61	9.34 ± 0.80
Pb*	µg/kg	19.53 ± 0.89	21.48 ± 5.91	6.70 ± 1.10

*All elements were acquired in He mode (enhanced He for P, S, As, and Se). *Pb was measured as the sum of the three most abundant isotopes, 206, 207, and 208.*

Quantitative results and spike recoveries for cell-based samples

Method blank, quantitative results for liquid cell culture media, spent media, and conditioned spent media samples, and spike recovery data (FMB and FAP) are given in Tables 7, 8, and 9. Differences in the concentration of some elements measured in the same sample types (Table 8 and 9) were observed since the samples were from different batches of the cell culture media. The FBS in the media is a likely source of variation (for example, the high concentration of Pb in Sample 3), as it is a complex, mostly undefined, and variable product, which works well for cell culture. Research is underway to find a cost-effective way to replace FBS with plant-based ingredients for cultivated meat cell cultures.

To test for nonspectral interferences (matrix effects), two FMBs were prepared by spiking the blank at 10, 1000, or 4000 ppb. The 1000 ppb-level spikes were used for Na, Mg, Ca, and Fe and the 4000 ppb spikes for P, S, and K. The low-level spike was used for the remaining trace elements. The FMB was analyzed periodically throughout the entire sample run. All recoveries were within the EAM 4.7 method acceptable % recovery range of 90–110%, as shown in Table 7.

A spike recovery (FAP) test was carried out to check the accuracy of the 7850 ICP-MS method for the analysis of liquid cell culture media. Eight culture media samples were spiked with trace elements at 10 ppb and major elements at 1000 ppb. The recoveries for all elements in the cell culture media samples were within the EAM 4.7 method QC criteria of ±20%, as shown in Tables 7 to 9.

Table 7. Method blank, quantitative results (n=9), and spike recoveries for media liquid samples.

		Method Blank			Liquid Cell Culture Media			
					Sample 1		Sample 2	
	Conc Unit	Method Blank Conc	Recovery Low Spike (%)	Recovery High Spike (%)	Culture Media Liquid (No Cells)	Recovery (%)	Culture Media Liquid (No Cells)	Recovery (%)
11 B	µg/kg	7.472	-	93	<LOD	103	<LOD	96
23 Na	mg/kg	15.98	-	108	2152	*	9913	*
24 Mg	mg/kg	<LOD	-	105	11.63	104	36.34	104
27 Al	µg/kg	0.448	106	101	<LOD	113	<LOD	109
31 P	mg/kg	<LOD	-	96	32.77	104	137.42	106
34 S	mg/kg	<LOD	-	**	<LOD	101	<LOD	98
39 K	mg/kg	<LOD	-	105	166.8	99	691.5	80
43 Ca	mg/kg	<LOD	-	102	30.92	102	125.1	99
47 Ti	µg/kg	<LOD	102	-	<LOD	98	<LOD	102
51 V	µg/kg	<LOD	106	-	<LOD	102	<LOD	101
52 Cr	µg/kg	<LOD	106	-	<LOD	101	<LOD	100
55 Mn	µg/kg	<LOD	-	98	<LOD	103	<LOD	101
56 Fe	mg/kg	0.859	-	106	0.380	107	2.044	107
59 Co	mg/kg	<LOD	105	-	<LOD	101	0.065	101
60 Ni	µg/kg	<LOD	106	-	<LOD	102	<LOD	104
63 Cu	µg/kg	0.053	-	107	<LOD	105	0.104	105
66 Zn	µg/kg	<LOD	-	103	<LOD	104	1.265	112
75 As	µg/kg	<LOD	104	-	<LOD	101	<LOD	102
78 Se	µg/kg	<LOD	104	-	<LOD	99	<LOD	96
88 Sr	µg/kg	<LOD	95	-	<LOD	105	<LOD	106
95 Mo	mg/kg	<LOD	105	-	<LOD	102	0.011	103
107 Ag	µg/kg	<LOD	-	102	<LOD	81	<LOD	82
111 Cd	µg/kg	<LOD	106	-	<LOD	105	<LOD	106
118 Sn	µg/kg	3.580	91	-	<LOD	100	<LOD	103
121 Sb	µg/kg	<LOD	104	-	<LOD	106	<LOD	106
137 Ba	µg/kg	<LOD	103	-	<LOD	104	<LOD	104
201 Hg	µg/kg	<LOD	101	-	<LOD	115	<LOD	101
205 Tl	µg/kg	<LOD	98	-	<LOD	104	<LOD	105
Pb***	µg/kg	<LOD	104	-	<LOD	104	<LOD	105

*All elements were acquired in He mode (enhanced He for P, S, As, and Se). *Spike level too low compared to native concentration, **below calibration range. ***Pb was measured as the sum of the three most abundant isotopes, 206, 207, and 208.*

Table 8. Quantitative results (n=9) and spike recoveries for spent media liquid samples incubated for 21 days.

		Spent Media After Being Used to Culture Primary Embryonic Chicken Muscle Precursor Cells for 21 days					
	Conc Unit	Sample 6	Recovery (%)	Sample 7	Recovery (%)	Sample 8	Recovery (%)
11 B	µg/kg	<LOD	96	<LOD	103	<LOD	102
23 Na	mg/kg	1221	*	2228	*	6541	*
24 Mg	mg/kg	8.333	106	11.88	106	27.93	106
27 Al	µg/kg	<LOD	94	<LOD	111	<LOD	108
31 P	mg/kg	24.35	105	31.08	106	89.59	107
34 S	mg/kg	<LOD	100	<LOD	102	<LOD	104
39 K	mg/kg	102.5	82	154.1	104	446.7	108
43 Ca	mg/kg	48.99	108	25.09	105	79.14	108
47 Ti	µg/kg	<LOD	104	0.062	102	0.081	103
51 V	µg/kg	<LOD	102	<LOD	101	<LOD	102
52 Cr	µg/kg	<LOD	102	<LOD	103	<LOD	102
55 Mn	µg/kg	<LOD	104	0.014	103	<LOD	103
56 Fe	mg/kg	14.03	107	0.371	107	0.837	107
59 Co	mg/kg	<LOD	102	<LOD	101	<LOD	102
60 Ni	µg/kg	0.030	102	<LOD	102	<LOD	102
63 Cu	µg/kg	1.145	109	<LOD	106	<LOD	105
66 Zn	µg/kg	5.212	98	<LOD	105	0.423	105
75 As	µg/kg	3.724	100	6.749	100	6.463	100
78 Se	µg/kg	<LOD	98	<LOD	98	<LOD	99
88 Sr	µg/kg	<LOD	105	<LOD	104	<LOD	104
95 Mo	mg/kg	<LOD	102	<LOD	102	<LOD	103
107 Ag	µg/kg	<LOD	80	<LOD	80	<LOD	80
111 Cd	µg/kg	<LOD	104	<LOD	104	<LOD	104
118 Sn	µg/kg	<LOD	100	<LOD	99	<LOD	99
121 Sb	µg/kg	<LOD	104	<LOD	105	<LOD	105
137 Ba	µg/kg	<LOD	105	<LOD	104	<LOD	103
201 Hg	µg/kg	<LOD	117	<LOD	114	<LOD	119
205 Tl	µg/kg	3.813	104	4.235	104	6.944	104
Pb	µg/kg	228.7	100	0.058	104	<LOD	105

*All elements were acquired in He mode (enhanced He for P, S, As, and Se). *Spike level too low compared to native concentration. **Pb was measured as the sum of the three most abundant isotopes, 206, 207, and 208.*

Table 9. Quantitative results (n=9) and spike recoveries for conditioned media liquid samples incubated for 14 days.

		Spent Media After Being Used to Culture Primary Embryonic Chicken Muscle Precursor Cells for 14 days					
	Conc Unit	Sample 6	Recovery (%)	Sample 7	Recovery (%)	Sample 8	Recovery (%)
11 B	µg/kg	<LOD	103	<LOD	103	<LOD	104
23 Na	mg/kg	3457	*	1774	*	2378	*
24 Mg	mg/kg	18.08	107	9.834	106	14.39	106
27 Al	µg/kg	<LOD	114	<LOD	105	<LOD	109
31 P	mg/kg	46.33	109	23.92	106	39.31	107
34 S	mg/kg	<LOD	105	<LOD	103	<LOD	106
39 K	mg/kg	232.3	106	126.9	105	159.7	104
43 Ca	mg/kg	42.79	110	20.72	104	32.27	104
47 Ti	µg/kg	<LOD	103	<LOD	104	<LOD	105
51 V	µg/kg	<LOD	103	<LOD	102	<LOD	102
52 Cr	µg/kg	<LOD	102	<LOD	103	<LOD	101
55 Mn	µg/kg	<LOD	104	<LOD	103	<LOD	102
56 Fe	mg/kg	0.683	108	0.280	107	0.794	106
59 Co	mg/kg	<LOD	103	0.013	102	0.015	101
60 Ni	µg/kg	<LOD	104	<LOD	104	<LOD	104
63 Cu	µg/kg	0.046	107	0.039	107	0.033	106
66 Zn	µg/kg	0.404	110	<LOD	106	<LOD	107
75 As	µg/kg	<LOD	103	<LOD	101	<LOD	100
78 Se	µg/kg	<LOD	101	<LOD	98	<LOD	99
88 Sr	µg/kg	<LOD	107	<LOD	106	<LOD	105
95 Mo	mg/kg	<LOD	104	<LOD	104	<LOD	103
107 Ag	µg/kg	<LOD	82	<LOD	82	<LOD	81
111 Cd	µg/kg	<LOD	106	<LOD	105	<LOD	106
118 Sn	µg/kg	<LOD	103	<LOD	102	<LOD	99
121 Sb	µg/kg	<LOD	106	<LOD	106	<LOD	104
137 Ba	µg/kg	0.407	106	<LOD	104	<LOD	104
201 Hg	µg/kg	<LOD	110	<LOD	108	<LOD	111
205 Tl	µg/kg	3.117	106	2.220	105	2.659	105
Pb**	µg/kg	0.381	106	<LOD	105	<LOD	105

*All elements were acquired in He mode (enhanced He for P, S, As, and Se). *Spike level too low compared to native concentration. **Pb was measured as the sum of the three most abundant isotopes, 206, 207, and 208.*

ISTD recovery (%)

The analytical sequence outlined in Figure 1 was analyzed repeatedly over 48 hours. All the ISTD recovery plots were within ±20%, with no internal standard failures throughout the run, meeting the criteria specified in EAM 4.7 (Figure 3). The results demonstrate the robustness of the 7850 ICP-MS plasma and high matrix tolerance of the system with UHMI over long runs.

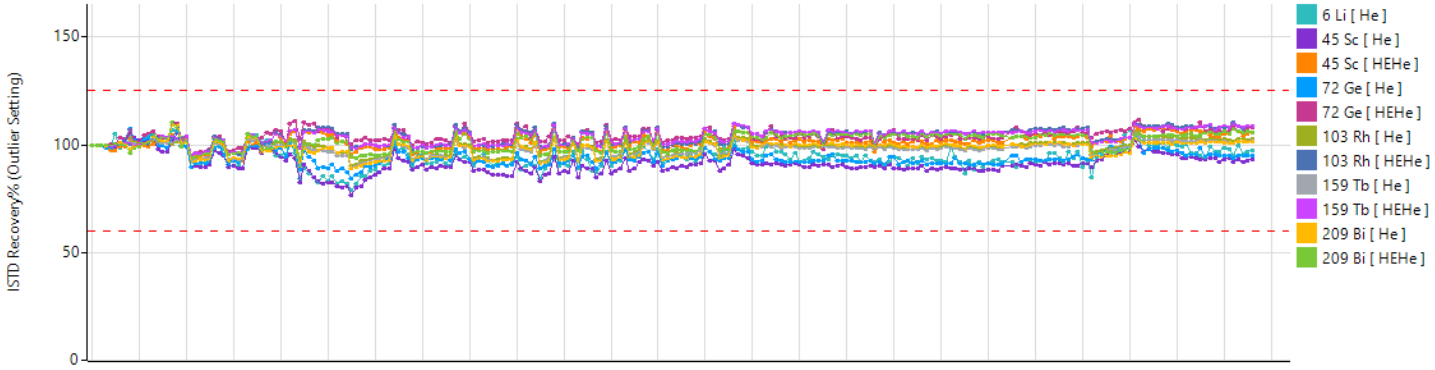

Figure 3. Stability of ISTD measurements over 48 hours. The ISTD recoveries have been normalized to the calibration blank for all samples.

IntelliQuant data

When an analyst develops a quantitative method using an ICP-MS MassHunter preset method, an IntelliQuant Quick Scan acquisition is predefined in the He mode tune step. No special setup or separate calibration is needed for IntelliQuant, simplifying the analysis. IntelliQuant automatically acquires full mass-spectrum data in every sample with only two seconds additional measurement time, allowing the analyst to quickly see which elements are present in the samples. Because IntelliQuant data is acquired in He collision cell mode, analytes are free from common polyatomic ion overlaps, ensuring the quality of the data.

In this study, IntelliQuant data was acquired for each plant-based food sample and SRM with the 7850 ICP-MS operating in He mode. The data can be displayed in a periodic table heat map view, as shown for the plant-based "minced beef" sample in Figure 4. The color intensity heat map shows the approximate concentration of up to 78 elements in each sample, with a darker color indicating a higher concentration of an element. The IntelliQuant data is a quick and simple way to get an overview of the elemental content of a sample and identify the presence of any unexpected elements.

Figure 4 shows that the plant-based "minced beef" sample contained a relatively high concentration of Rb. Rb wasn't calibrated as part of the quantitative study, so the natural isotope template feature of IntelliQuant was used to check the Quick Scan spectrum to confirm its identity. Figure 5 shows a good fit to the natural isotope template for Rb, confirming its presence in the sample.

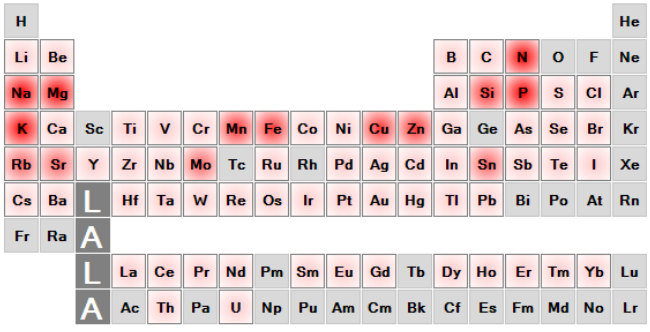

Figure 4. Periodic table heat map view of ICP-MS IntelliQuant data acquired for the plant-based "minced beef" sample.

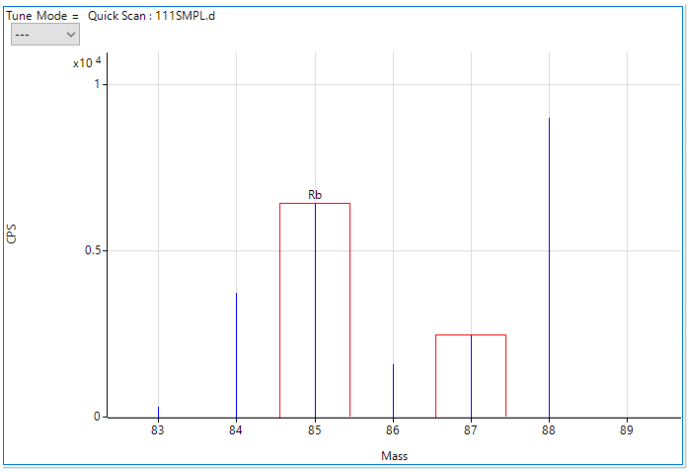

Figure 5. The unexpectedly high concentration of Rb in the plant-based minced beef sample was confirmed using the isotope template fit function in the IntelliQuant Quick Scan mass spectrum.

Conclusion

The Agilent 7850 ICP-MS was used to analyze 30 elements in a range of plant-based protein foods and 29 elements in a range of cell culture media. The analysis was done in accordance with US FDA EAM method 4.7 for food and related products and included the 12 elements specified in the 4.7 method. All the food samples were prepared in the same batch using a single microwave digestion method, while the cell media samples were simply diluted before analysis.

The 7850 ICP-MS method was predefined based on a previous EAM 4.7 food analysis batch, and the instrument was autotuned, saving development time. All elements were measured using a single data acquisition mode, with effective removal of polyatomic interferences ensured by operating the ORS^4 collision cell in He-KED mode. Also, as part of the quantitative method using He-KED mode, IntelliQuant data was acquired for each sample. The IntelliQuant data for the plant-based "minced beef" sample was displayed as a heat map of the periodic table, showing approximate concentration ranges for each of the measured elements. IntelliQuant's isotopic template was used to confirm the identity of uncalibrated elements such as rubidium in the plant-based "minced beef" sample.

The accuracy of the quantitative method was evaluated by analyzing four food-based SRMs and conducting a spike recovery test of the plant-based "beef" sample. Excellent recoveries were achieved for both tests, within EAM 4.7 method QC criteria of ±10% and ±20%, respectively. The 7850 ICP-MS exceeded the nominal detection limit requirements specified in the EAM method. Also, using the UHMI aerosol dilution technology, the 7850 showed excellent stability over a 48-hour ISTD recovery run, demonstrating the robustness of the method.

The same 7850 ICP-MS method was also used to analyze various liquid cell culture media samples and spiked samples. Good spike recovery data was achieved for eight samples, confirming the suitability of the method to support the development of cultured meat products—a growth market in the food industry.

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