Sample Preparation and Analysis of Dietary Supplements Using Sequential Microwave Digestion

Abstract

Elemental analysis of dietary supplements is necessitated by both governmental regulation and safety of potential consumers. It is important that there is a safe, rapid, and efficient way to digest these supplements to provide a more accurate analysis via Inductively Coupled Plasma Mass Spectrometry (ICP-MS). The BLADE™ sequential microwave digestion system from CEM Corporation can safely operate at the temperatures and pressures required to provide a rapid and efficient digestion of these materials. A variety of dietary supplements, including Standard Reference Materials (SRMs), were digested on the BLADE for analysis by ICP-MS. The method was based on USP 232/233 with a focus on Class 1 (As, Cd, Hg, Pb) and Class 2a (Co, Ni, V) elements. Recovery values for SRMs and spiked dietary supplements were excellent, demonstrating the capabilities of the BLADE with these types of materials. In addition, all samples tested fell well below the permitted daily exposure limits outlined in USP 232.

Introduction

Growing consumer demand placed on health and nutrition has led to a plethora of dietary supplements available in the consumer market. The USFDA (United States Food and Drug Administration) defines these dietary supplements as items different than consumed foods with intent to supplement the diet through either vitamins, minerals, botanicals and herbs, botanical compounds, amino acids, or live microbials[1]. Supplements are produced in varied forms for ease of ingestion including gel capsules, dissolvable, gummies, and powders. Dietary supplements are gaining significant traction with consumers, with approximately 60% of Americans[2] using some sort of supplement from the over 50,000 available[3].

As with all ingested foods, heavy metals and elemental impurities are a cause for concern due to their negative impacts on human health. Elemental impurities come from a variety of sources during the production of dietary supplements, occurring either from the raw materials or inadvertent introduction during manufacturing. Under the USFDA Food, Drug, and Cosmetic Act, many criteria are used for regulating dietary supplements, including prohibition of adulterated or misbranded products, as well as, elemental impurities. It is of the utmost importance to ensure consumer safety by analyzing and monitoring the elemental levels present in dietary supplements.

In this study, we utilized the BLADE sequential microwave digestion system to digest different forms of multivitamins, dissolvable tablets, and a fish oil capsule. Samples were chosen to encapsulate a large range of capsule materials, as well as precursor materials.

Materials and Methods

Instrumentation

The samples discussed were all digested using the BLADE sequential microwave digestion system. The BLADE provides sequential digestion of samples with automatic loading and unloading using a robotic arm. The system uses quartz vessels and optional Teflon® liners with a two-piece snap cap. Samples are heated to temperature using microwave energy and stirred with stir bars to ensure even digestion. After digestion, samples are cooled, unloaded from the cavity, and the next sample is loaded following the same sequence until the entire queue is finished. The sequential format of the BLADE allows for a variety of acids and digestion temperatures to be used in a batch. Samples can be observed during digestion with the built-in camera.

Sample Preparation

USP 232/ICH Q3D Class 1, 2a and 2b Oral Elemental Impurities standards purchased from Inorganic Ventures were used to spike the acid blanks and fish oil at 0.5 J, 1.0 J and 1.5 J for oral consumption of fish oil. The samples analyzed in this study included various dietary supplements meant to be taken orally. SRMs, Multielement Tablet (NIST SRM 3294) and Gingko Biloba (NIST SRM 3246) were used as validation samples. Other samples digested were multivitamin gummy and tablet, dissolvable tablet, and fish oil (all purchased at a local store). All tablet-style samples were ground to a fine powder prior to digestion and the fish oil capsule was cut open to release the housed oil prior to digestion.

The acid matrix used to digest 0.5 g of each sample is listed in **Table 1** (on page 2). All samples were digested at 220 °C and run in triplicate.

Table 1. Acid Matrix Used to Digest Each Sample

Sample	Acid Matrix
NIST SRM 3294 – Multielement Tablet	5mL HNO_3 + 1mL HCl + 0.5mL HF
NIST SRM 3246 – Gingko Biloba	5mL HNO_3+ 1mL HCl + 0.5mL HF
Multivitamin Gummy	5mL HNO_3 + 1mL HCl + 0.5mL HF
Multivitamin Tablet	5mL HNO_3 + 1mL HCl + 0.5mL HF
Dissolvable Tablet	5mL HNO_3 + 1mL HCl
Fish Oil	5mL HNO_3 + 1mL HCl + 5mL DI H_2O

Analysis

Samples were analyzed using an Agilent 7850 ICP-MS (Agilent Technologies, Santa Clara, CA). The conditions used for the majority of elements are listed in **Table 2**. For certain elements, separate tuning modes were used to minimize interferences. Elements analyzed were based upon elements certified in the SRMs and standards, including class 1 (As, Cd, Hg, Pb) and 2a (Co, Ni, V) elements, according to USP 232/233. A modified methodology for USP 232/233 was used for analysis.

Table 2. Instrument Conditions of Agilent 7850 ICP-MS

Parameters	Values
RF Power (kw)	1550
Sampling Depth (nm)	8
Carrier Gas (L/min)	1.05
Helium Cell Gas (mL/min)	5.0
Energy Discrimination	5.0

Results

Acid blank values are presented in **Table 3**. Element concentrations in the acid blank and unspiked fish oil sample, were negligible or not detected. Percent recovery data for an acid blank spiked at 0.5 J, 1.0 J, and 1.5 J, as well as a fish oil sample spiked at 1.0 J are graphed in **Figure 1**. All recovery values for the spiked samples were between 90-115%. Acid blank and spiked data were utilized for verification of the analysis on the ICP-MS.

Table 3. Recoveries of Acid Blanks and Unspiked Fish Oil, ppb (n=3)

Element	(5:1) HNO_3:HCl	(5:5:1) HNO_3:H_2O:HCl w/0.5g Fish Oil
As	n.d.	n.d.
Au	0.0006667	0.0006667
Cd	0.001667	0.004000
Co	0.004767	0.02100
Hg	0.01100	0.007000
Ir	n.d.	n.d.
Ni	0.2670	0.2343
Pb	0.01567	0.009667
Pd	n.d.	n.d.
Pt	0.001667	0.003667
Rh	n.d.	n.d.
Ru	n.d.	0.0003333
Se	n.d.	n.d.
Tl	0.008000	0.01200
V	n.d.	n.d.

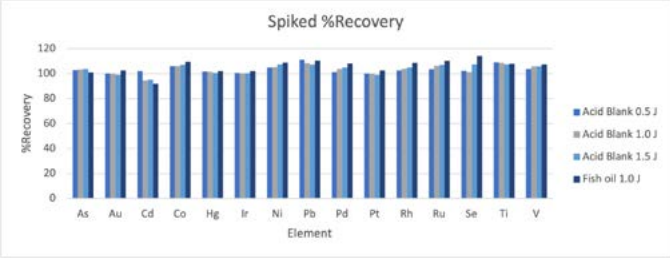

Figure 1. Recovery for Spiked Acid Blanks and Spiked Fish Oil (n=3)

Elemental concentrations and percent recovery values for the NIST SRMs, Multielement Tablet (SRM 3294), and Gingko Biloba (SRM 3246) are presented in **Table 4** (on page 3). Recoveries for both SRMs were found to be within the 90-115% range for the certified elements, well within the criteria specified in USP 233.

Table 4. Average Elemental Concentrations (ppb) and Percent Recovery Values for the Certified Elements in the NIST SRMs (n=3)

	As	Cd	Co	Hg	Ni	Pb	Se	V
Multielement Tablet	148.6	83.95	882.3		8523	245.5	16180	8503
Expected	132.0	80.15	810.0		8430	272.7	17420	8000.
% Recovery	112.6	104.7	108.9		101.1	90.02	92.91	106.2
Ginko Biloba		22.00		24.16		1018		
Expected		20.80		23.08		995.0		
% Recovery		105.8		104.7		102.3		

The elemental concentrations of the store-bought dietary supplements digested are presented in **Table 5**. The average elemental concentrations for USP 232/233 Class 1 and 2a elements were found to be below the PDE for the samples tested.

Table 5. Average Elemental Concentrations (ppb) of Dietary Supplements (n=3)

Element	Dissolvable Tablet	Multivitamin Tablet	Multivitamin Gummy	Fish Oil
As	0.5090	201.9	27.87	n.d.
Cd	11.96	322.8	11.03	0.2353
Co	0.8967	1232	303.1	0.002333
Hg	2.569	2.145	2.273	1.201
Ni	4.977	2596	258.4	4.302
Pb	115.1	147.3	33.62	0.1273
V	n.d	1194	398.9	n.d.

Conclusion

Proper monitoring and analysis of elemental impurities in dietary supplements is imperative to ensuring consumer safety. Efficient sample preparation providing homogeneous solutions for analysis plays a vital role in this process. By utilizing the BLADE sequential microwave digestion system, multiple dietary supplements were digested and analyzed for their elemental content. Strong recoveries from both NIST SRMs and spiked fish oil samples showed the suitability of digestion and analysis protocols. Since the BLADE digests one sample at a time, the BLADE methods are more amenable to rush samples and rapid method optimization.

In addition, the BLADE methods often use less acid in comparison to batch microwave digestion and can complete a run in a fraction of the time.

References

[1] U.S. Food and Drug Administration. (June 2, 2022). FDA 101: Dietary Supplements. https://www.fda.gov/consumers/consumer-updates/fda-101-dietary-supplements (accessed August 9, 2023).

[2] Mishra, S.; Stierman, B.; Jaime J. Gahche, J.; Potischman, N. Dietary Supplement Use Among Adults: United States, 2017–2018. *Centers for Disease Control and Prevention* [Online] February, 2021, NCHS Data Brief No. 399. https://www.cdc.gov/nchs/products/databriefs/db399.htm (accessed August 9, 2023).

[3] Cohen, P.A. Assessing Supplement Safety–the FDA's Controversial Proposal. *The New England Journal of Medicine*. [Online] **2012**; 366: 389–391 https://www.nejm.org/doi/full/10.1056/NEJMp1113325 (accessed August 9, 2023).

Find a Local Contact

cem.com/contact