

## DIRECT Hg ANALYSIS IN MICROALGAE-BASED SUPPLEMENTS USING DMA-80 *evo*

### Assessing Mercury Contamination in Microalgae-Based Supplements: What to Expect

#### I SUMMARY

Microalgae- and algae-based supplements have gained significant popularity in recent years due to their impressive nutrient profile, which is rich in proteins, vitamins, and antioxidants. The United Nations World Food Conference has notably recognized *Spirulina platensis* as “food of the future”, underscoring its excellent nutritional value and minimal resource requirements for cultivation. However, concerns have been raised regarding the quality of these products due to contamination with heavy metals, inorganic arsenic, and cyanotoxins, which can bring healthy risks. Microalgae have a special ability to absorb and accumulate heavy metals, including mercury, from their surrounding aquatic environment; they have cell walls containing specific functional groups, such as

carboxyl, hydroxyl, and sulfhydryl groups, which allow these photosynthetic organisms to effectively bind with harmful substances in their growth environments [3]. Elements such as mercury are known to bioaccumulate in microalgae and can have toxic effects on the nervous system.

Their remarkable ability to accumulate these substances is currently being examined in different studies for industrial wastewaters cleaning applications to understand how it can be exploited.

Given the increase and spread of these microalgae-derived products as food supplements in the population, understanding the risks associated with mercury contamination has become critical. The challenges in mercury determination are well known, but the major concern is usually sample preparation, resulting in



time-consuming and expensive techniques. Direct Mercury Analysis technology (DMA) allows the analysis of any matrix without any pre-treatment or chemical additions in as few as 7 minutes.

## I EXPERIMENTAL

### INSTRUMENTATION

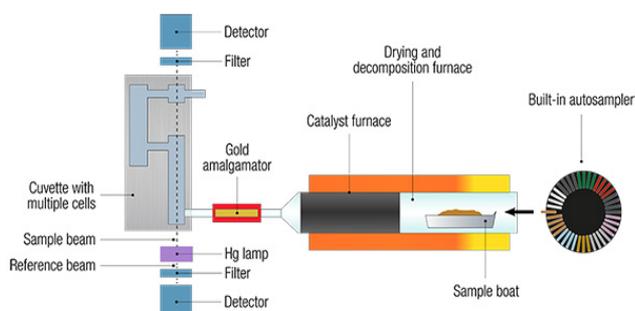
- Milestone DMA-80 *evo* Tricell Double-Beam
- Milestone Air Compressor
- Quartz boats
- Analytical balance



Milestone's DMA-80 *evo* with EasyCONTROL terminal.

### PRINCIPLE OF OPERATION

Samples were firstly homogenized, then weighed on an analytical balance and lastly introduced into quartz boats for the analysis on the DMA-80 *evo*.



Summary of the DMA-80 Dualcell operating principle.

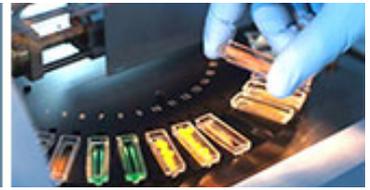
Controlled heating stages are implemented to first dry and then thermally decompose the sample introduced into the quartz tube. A continuous airflow or oxygen flow carries the decomposition fumes through a hot catalyst where interferences (halogens, sulfur oxides, etc....) are trapped. All mercury species then are reduced to elemental mercury (Hg<sup>0</sup>) and are carried along with reaction gases to a gold amalgamator where the elemental mercury is selectively trapped. All other vapors are flushed from the system by the continuous flow of gas. The amalgamator is subsequently heated and releases all the trapped mercury into the atomic absorption spectrophotometer (AAS). Absorbance is measured at 253.7 nm as a function of mercury content.

### CALIBRATION

Since mercury is thermally released from the sample, measurements are matrix-independent, and instrument calibrations are long-lasting.

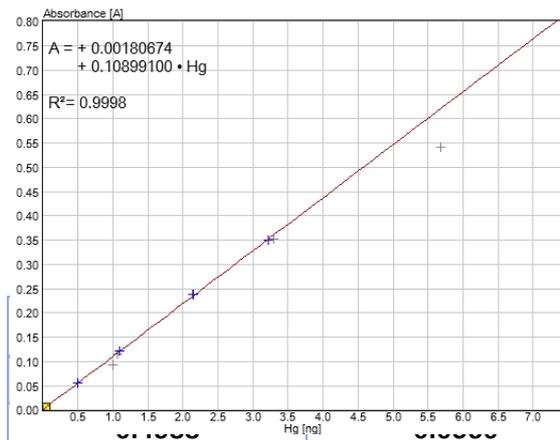
Liquid calibration was prepared using a commercially available NIST Hg(NO<sub>3</sub>)<sub>2</sub> in HNO<sub>3</sub> traceable Mercury Standard Solution of 1000 mg/L Hg.

Working standards from 1 to 10000 ppb were prepared and preserved with 2% ultrapure HCl for mercury stability. Calibration curves were created on the system by injecting increasing volumes of standards (from 1ppb to 10 ppm) directly into dedicated quartz boats, thus covering the range of 0-1000 ng of mercury to calibrate all the different cells in the system.



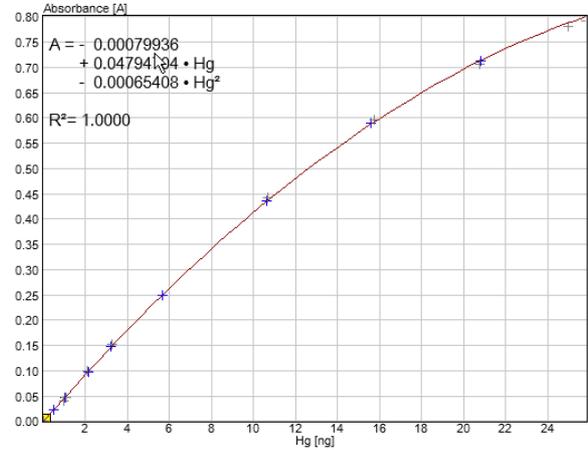
For this calibration, at least five data points were collected for each cell following thorough system cleaning. Each measurement was performed in duplicate to minimize experimental error, and only the most accurate value was retained to build the calibration curve. Once done with the calibration, the calibration curve was checked using a certified reference material (CRM) that has a similar matrix to the chosen samples: IAEA-450, that contains different concentrations of heavy metals and mercury (104 ppb).

**Cell 0 calibration curve:**



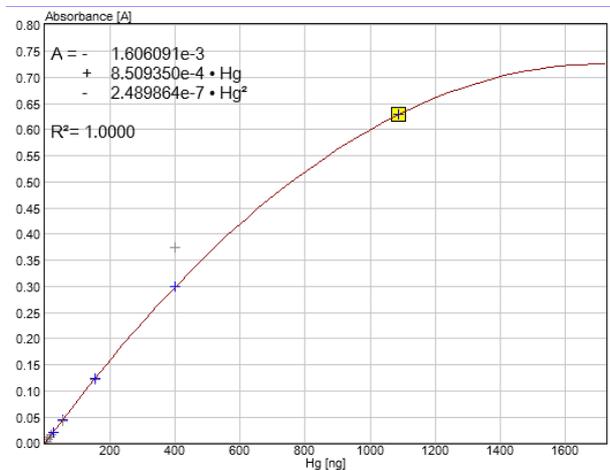
0.0000	0.0004
1.0640	0.1221
2.1506	0.2394
3.2198	0.3504

**Cell 1 calibration curve:**



Hg (ng)	A <sup>^</sup>
0.0000	0.0004
0.4988	0.0231
1.0640	0.0475
2.1506	0.0989
3.2198	0.1493
5.6851	0.2487
10.6546	0.4354
15.6038	0.5895
20.7950	0.7128

**Cell 2 calibration curve:**

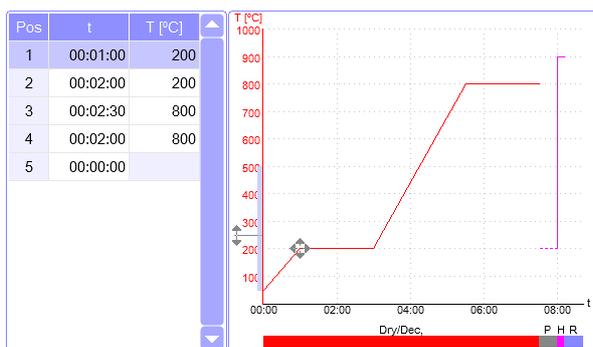




Hg (ng)	A <sup>^</sup>
25.8250	0.0212
55.7006	0.0450
156.302	0.1236
400.704	0.3002
1086.39	0.6289

### OPERATING CONDITIONS

The method used in this study is the one developed for organic samples. There are two main heating steps: the first at 200°C degrees for the dehydration of the sample.



Method developed for organic samples

and the second one at 800°C degrees for the complete decomposition of the organic substances, for a total time of seven and half minutes. The catalyst temperature is fixed and settled on 600°C degrees; also, the amalgamator heating is settled on 850/900°C degrees.

### RESULTS AND DISCUSSION

Eight microalgae samples were analysed, with three repetitions each to increase the

accuracy of the analytical data. In the table below are shown the weights of the analysed samples, their concentration in ppb ( $\mu\text{g}/\text{Kg}$ ) and the standard deviation of the measurements.

Spirulina microalgae (*Arthrospira Platensis*) is one of the most popular supplement products in recent years and can easily be found in local pharmacies or from online retailers. It is a single-celled blue algae with a narrow, elongated shape that is no more than half a millimetre long. Chlorella (*Chlorella Pyrenoidosa*) is also a small single-celled alga, like Spirulina; both live in fresh water. Three different varieties of spirulina and two variants of chlorella were analysed (shown as 1, 2 or 3 to maintain the commercial brand hidden).

Klamath AFA algae (*Aphanizomenon Flos-aquae*) is a cyanobacterium considered to be a microalga that specifically grows naturally in freshwater lakes located in the mountainous regions of Oregon.

Fucus is an iodine-rich brown seaweed characteristic of the colder areas of the Mediterranean Sea and the Atlantic Ocean. Kelp is a brown seaweed that grows on the shores of the oceans. Both Kelp and Fucus are seaweeds, which do not come from fresh water but from the ocean. This should, in theory, make them richer in mercury due to their place of origin.



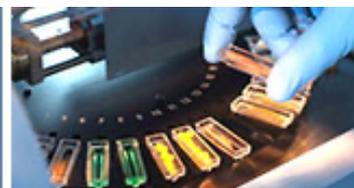
Table 1: Samples analysis, with 3 repetitions each. Reported the mercury content absolute value and the concentration ( $\mu\text{g}/\text{Kg}$ ) based on the sample amount.

Sample ID	S. weight (g)	Measured Hg ( $\mu\text{g}/\text{Kg}$ )	Mean Hg content (n=3) ( $\mu\text{g}/\text{Kg}$ )	Std Dev. ( $\mu\text{g}/\text{Kg}$ )	RSD%	of
Spirulina	0.0630	9.2712	8.9766	0.3438	3.82	
	0.0640	8.5989				
	0.0628	9.0598				
Spirulina 2	0.0591	5.1354	4.9028	0.2742	5.59	
	0.0627	4.9726				
	0.0627	4.6004				
Spirulina 3	0.0633	1.7346	1.7229	0.1105	6.41	
	0.0589	1.6071				
	0.0540	1.8271				
Chlorella	0.0610	1.4029	1.4642	0.0552	3.77	
	0.0550	1.4800				
	0.0434	1.5099				
Chlorella 2	0.0665	0.5509	0.5542	0.0254	4.58	
	0.0610	0.5306				
	0.0570	0.5810				
Klamath AFA	0.0493	5.5870	5.5041	0.1123	2.04	
	0.0538	5.3763				
	0.0515	5.5491				
Kelp	0.0645	18.1190	17.9806	0.7416	4.12	
	0.0530	18.6433				
	0.0800	17.1795				
Fucus	0.0549	7.0646	6.8037	0.2269	3.33	
	0.0652	6.6525				
	0.0659	6.6941				

As expected, algae from oceanic source contain more mercury than lake ones. However, the mercury concentration is far under the safety limit (100 ppb). Nevertheless, it must be taken into account that, beyond the nutritional value, some mercury contamination is detected in all analyzed supplements. Consider that, for example, for sample Spirulina 1 the suggested daily intake is four tablets and one tablet is nearly 0.50 g; so, the amount

ingested mercury is near 18 ng/day, that should not be underestimated.

After analysing all the samples, all of them were reintroduced into the DMA-80 with the addition of a spike of mercury standard solution (100 ppb) previously used for instrument calibration. This is helpful for assessing the precision and accuracy of DMA performances on this kind of samples.



Sample ID	Sample weight (g)	Spike (mL)	Expected Hg (spike + sample) (ng)	Measured Hg (spike + sample) (ng)	% Spike recovery
Spirulina	0.0585	0.1140	11.40 + 0.5251	11.47	96.2
Spirulina 2	0.0725	0.1140	11.40 + 0.3555	12.34	95.3
Spirulina 3	0.0577	0.1140	11.40 + 0.0994	11.57	99.4
Chlorella	0.0523	0.1060	10.60 + 0.0766	11.05	96.6
Chlorella 2	0.0636	0.1060	10.60 + 0.0352	11.06	96.2
Klamath AFA	0.0590	0.1070	10.70 + 0.3247	11.38	96.9
Kelp	0.0382	0.1070	10.70 + 0.6869	11.59	98.2
Fucus	0.0590	0.1080	10.80 + 0.4014	11.75	95.3

Table 2: Data for direct determination of mercury in spiked supplements samples by means of direct mercury analyzer DMA-80; Spiked nearly 0.100 mL of 100 ppb standard.

All spike recoveries were over 95% and the medium recovery is near 97%. This shows the DMA performances and capabilities: the system was found to be very accurate and matrix-independent. Despite analysing different algae and combining solid (algae) and liquid (spike) samples, the results stand with a very high recovery of mercury.

## CONCLUSION

This study shows how the DMA-80 *evo* tricell addresses the task along with a great

ease of use and a high productivity. The double beam feature enhances the performance of the DMA-80 at low range, such as 0,5 µg/Kg (0.03 ng), ensuring a performing mercury recovery.

The mercury concentration in all analysed supplements was under the legal limit; nevertheless, attention must be paid to overconsumption of this kind of supplements and their simultaneous daily intake.



## REFERENCES

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- [4] *Application of microalgae in remediation of heavy metal-contaminated soils and its stimulatory effect on wheat growth, Algal Research, Volume 88, June 2025, Jia Liu et*

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