C1_C0062: VALIDATION OF THREE METHODS PREPARED FOR INDUCTIVELY COUPLED PLASMA MASS SPECTROMETRY FOR DETERMINATION OF HEAVY METALS IN LICHEN

Duangkamon Sangiamdee,[1,]* Chutima Sriviboon,[1] Narong Jayasuta,[1] Kansri Boonpragob[2]
[1]Department of Chemistry, Faculty of Science, Ramkhamhaeng University, Bangkok 10240, Thailand
[2]Department of Biology, Faculty of Science, Ramkhamhaeng University, Bangkok 10240, Thailand
*e-mail: poochem1986@gmail.com

Abstract: Three methods for preparation of lichen sample including dry ashing, wet digestion and ultrasound-assisted extraction were optimized and validated. The best technique used for inductively coupled plasma mass spectrometry (ICP-MS) to determine heavy metals accumulated in the lichen was determined. The heavy metals, As, Cd, Co, Cr, Cu, Ni, Pb, V, Zn, Ti, Sb, Se and Mo in the lichen *Parmotrema tinctorum* were analyzed. The ICP-MS method was validated for limit of detection, limit of quantification, accuracy, precision and linearity. Sample preparation methods were validated in term of precision and spiked recovery. The precision of wet digestion method in seven replicates of 13 heavy metals had relative standard deviation ranged between 1.60–7.31% whereas dry ashing and ultrasound-assisted extraction methods were in the ranges 2.41- 43.86 % and 3.03–21.12 % respectively. The spike recovery of 40 ppb for all heavy metals determined by wet digestion method ranged 59.76–113.73 % where as dry ashing and ultrasound-assisted extraction methods were 28.26–122.15 % and 56.82–147.20 % respectively. The results showed that the wet digestion method was the best technique. The lichen samples collected from Queen Sirikit Botanic Garden at Chiang Mai Province were analyzed.

Introduction: Sample preparation is often considered a critical step in elemental analysis.[1] Lichen sample can be prepared by digestion of the organic matrix to obtain an aqueous solution suitable for analysis using a variety of instruments. Different approaches have been used for lichen digestion. Three methods for sample preparation are commonly used. First, dry ashing method, of which the ashing eliminates the effect of organic materials in mineral element determination. After relatively elevated temperatures (450-550 °C) in a muffle furnace, ash residue is derived, and then dissolved in an appropriate acid.[2] Second, wet digestion with oxidizing acid, which is the most common sample preparation. The method includes wet decomposition and dissolution of the organic and inorganic samples in open and closed system. The open system usually digests on hot plate or heating box.[3] Third, ultrasound-assisted extraction has become an important aspect of sample preparation in environmental, soil and food analysis. The samples can be processed rapidly, cheaply, accurately. In addition it is also safer as it involves neither high pressure nor high temperature. Reagent consumption and the acid waste generation are considerably less than microwave digestion, dry ashing and wet digestion procedures.[4]

Inductively coupled plasma-mass spectrometry (ICP-MS) was selected as the basis of the alternative method since it provides good sensitivity, requires minimal sample size, affords minimal elemental interferences and readily provides a means to perform rapid and automated multi-elemental analyses. Because ICP-MS provides the analyst with a wide range of element selectivity, the analyst may opt to include as many elements as possible when developing a given method.[5]

The aims of this work were to optimize and validate sample preparation performance, including dry ashing, wet digestion and ultrasound-assisted extraction method and, to determine heavy metals accumulated in lichen by ICP-MS.

Methodology: The three methods were used to prepare samples of the lichen *Parmotrema tinctorum* for optimization and validation. The wet digestion and ultrasound-assisted extraction conditions were employed by varying the parameter as shown in Table 1 and 2 respectively. The dry ashing method conditions were modified from Jiathanakul.[6] The optimized conditions were applied with lichen sample as following: First, dry ashing method: 0.2 g of grounded lichen samples were accurately weight in crucibles. Then, these samples were ashed in dry condition in muffle furnace for 3 hrs at 550 °C. Thereafter, the dry samples were digested by adding 4 ml of 6 M HNO_3 and the mixtures were heated at about 150 °C to dryness and 4 ml of 1 M HNO_3 were added and heated again. They were then cooled at room temperature before 1 ml of 0.1 M HNO_3 and 5 ml of DI water were added and stir for 10 min. The aliquots were then filtered through 0.2 µm nylon membrane filters into 10 ml volumetric flask and diluted with DI water. Second, wet digestion: 0.2 g of grounded lichen samples were accurately weight placed in a digestion tubes. Digestion with optimized parameter is shown in Table 1. The digested solutions were cooled at room temperature then filtered through 0.2 µm nylon membrane filters into 10 ml volumetric flask and diluted with DI water. Third, ultrasound-assisted extraction: 0.2 g of grounded lichen samples were accurately weight placed in a polypropylene tubes then added 5 ml of 1% HNO_3. Extraction by ultrasonic bath at room temperature with optimized parameter is shown in Table 2. The extract was filtered through 0.2 µm nylon membrane filters into 10 ml volumetric flask and diluted with DI water. These solutions were kept in glass bottles at 4 °C until analysis, which was performed by using an PerkinElmer's NexION 300Q Inductively Coupled Plasma Mass Spectrometry (ICP-MS). The operational parameters are described in Table 3. The lichen sampls used in this study is *Parmotrema tinctorum* collected from bark of trees at Queen Sirikit Botanic Garden at Chiang Mai Province. They were brought to laboratory at R.U. to prepare for heavy metals analysis by the suitable preparation methods chosen from the three procedures described earlier. Lichen samples were collected from 25 sites, and each sample was analyzed 3 times.

Table 1. Experimental conditions for the wet digestion method

Variable	Studied interval	Optimized parameter
Time (min)	1-5 hr	3 hr
HNO_3 (extractant) (%, v/v)	6M & conc.HNO_3	Conc.HNO_3
Volume HNO_3	2-10 ml	2 ml
Sample amount (g)	0.1-0.2 g	0.2 g

Table 2. Experimental conditions for the ultrasound-assisted extraction method

Variable	Studied interval	Optimized parameter
Sonication amplitude	50 Hz	50 Hz
Sonication time (min)	3-5 min	4 min
HNO_3 (extractant) (%, v/v)	1-2%	1%
Sample amount (g)	0.1-0.2 g	0.1 g

Table 3. The operational parameters used in ICP-MS

Characteristics	Instrument conditions	
Nebulizer Gas Flow	0.91	L/min
Auxiliary Gas Flow	1.30	L/min
Plasma Gas Flow	15.00	L/min
Detector Voltage	-11.00	Volts
ICP RF Power	1550.00	Watt
Analog Stage Voltage	-1750.00	Volts
Pulse Stage Voltage	1050.00	Volts

Results, Discussion and Conclusion: The results of an ICP-MS validation in terms detection limit, linearity accuracy and precision are shown in Table 4. The method was suitable for analysis trace metals in sample within range of ppt-ppb. It can be confirmed that an ICP-MS is an effective analytical tool for determination of elements in samples from different environmental condition. It has capability to scan all elements sequentially allowing rapid sample processing and suitable for the lichen sample. The validation results of dry ashing, wet digestion and ultrasound-assisted extraction methods for precision and spike recovery are shown in Table 5. The data from precision and recovery indicated that the wet digestion method should be choosen for analysis heavy metals in lichen samples by ICP-MS. Figure 1. showed heavy metal contents (μg/g dw) in lichen prepared by the three methods. It indicated that wet digestion gave the highest amount and precision of V, As, Se and Mo, the ultrasound-assisted extraction offered the highest amount of Co, Cu, Cd, Zn, Pb and Sb and the dry ashing provided the highest amount of Ti, Cr and Ni. The average amount of heavy metal, determined by wet digestion, accumulated in lichen at Queen Sirikit Botanic Garden are shown in Table 6.

Table 4. The limit of detection, limit of quantification, linearity, precision and accuracy of elements by ICP-MS

Metal	LOD (ppb)	LOQ (ppb)	Linearity	Precision (%RSD) N=10	Accuracy (40ppb)
Ti	0.036	0.119	0.9997	3.649	98.44
V	0.023	0.077	0.9984	3.485	97.89
Cr	0.069	0.229	0.9997	2.463	97.76
Co	0.016	0.053	0.9973	3.945	99.36
Ni	0.041	0.137	0.9999	4.866	98.22
Cu	0.063	0.209	0.9998	4.134	96.89
Zn	0.643	2.144	0.9987	4.660	97.15
As	0.002	0.006	0.9999	4.370	99.99
Se	0.022	0.074	0.9999	4.516	98.45
Mo	0.022	0.072	0.9999	4.846	99.19
Cd	0.016	0.053	0.9999	2.935	98.80
Pb	0.010	0.033	0.9999	3.589	99.89
Sb	0.001	0.003	0.9999	1.948	98.12

Table 5. The precision and spike recovery of 40 ppb of elements prepared by three methods (N = 7)

	dry ashing method			wet digestion method			ultrasound-assisted extraction method		
Metal	$\bar{X}$± SD (µg/g)	%RSD	%recovery	$\bar{X}$ ± SD (µg/g)	%RSD	%recovery	$\bar{X}$ ± SD (µg/g)	%RSD	%recovery
Ti	5.23±0.13	2.41	76.78	3.93±0.17	4.29	59.76	1.89±0.06	6.06	56.82
V	0.43±0.01	3.45	105.04	0.55±0.01	1.60	79.22	0.51±0.01	3.22	88.36
Cr	2.20±0.09	3.95	122.15	1.66±0.11	6.65	93.86	0.59±0.03	9.67	98.43
Co	0.10±0.04	37.05	72.95	0.16±0.005	2.99	101.43	0.23±0.003	3.03	113.13
Ni	1.81±0.11	6.11	49.76	1.68±0.08	4.77	94.00	1.78±0.05	5.99	117.13
Cu	3.94±0.51	13.06	76.46	3.92±0.14	3.47	95.94	5.69±0.21	7.21	125.17
Zn	25.25±1.08	4.27	44.23	29.60±1.53	5.16	102.58	51.30±1.99	7.76	96.12
As	0.09±0.004	4.42	41.16	0.27±0.01	2.88	104.28	0.09±0.001	3.11	112.77
Se	0.05±0.01	11.61	49.95	0.16±0.01	3.65	100.63	0.04±0.002	9.84	101.89
Mo	0.05±0.02	43.86	80.37	0.11±0.01	7.31	106.80	0.04±0.004	21.12	69.06
Cd	0.14±0.02	13.53	109.15	0.18±0.01	4.58	108.88	0.27±0.01	4.52	117.83
Pb	3.56±0.30	8.43	68.18	4.32±0.15	3.56	113.73	5.92±0.12	4.10	147.20
Sb	0.01±0.001	11.87	28.26	0.02±0.001	5.01	92.34	0.03±0.002	9.68	93.36

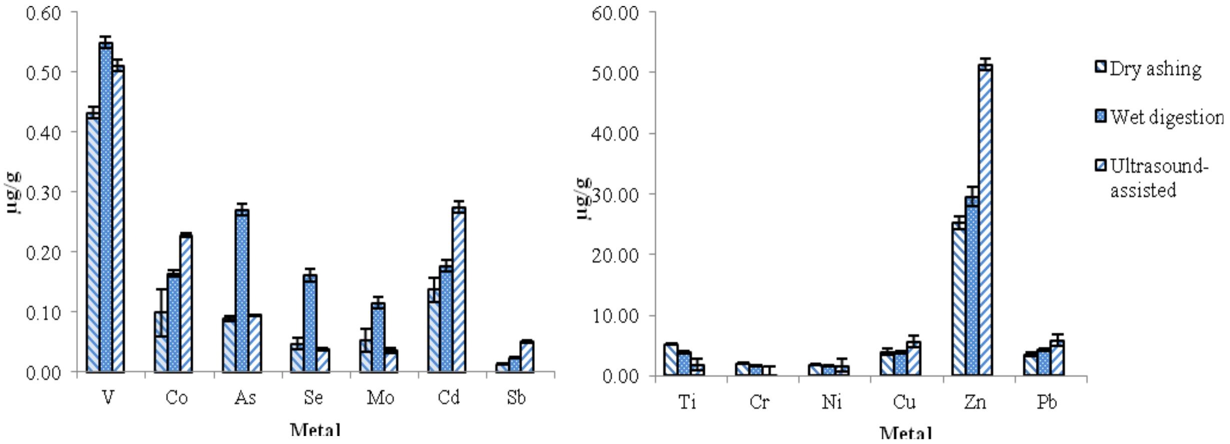

Figure 1. Heavy metal contents (µg/g dw) in lichen prepared by three different methods, n = 7.

Table 6. The average amount of heavy metal concentration (µg/g dw) and coefficient of variation in lichen *P. tinctorum* collected from Queen Sirikit Botanic Garden.

Metal	Queen Sirikit Botanic Garden			
	$\bar{x}$ ± SD	Max	Min	CV
Ti	4.67±2.02	10.03	1.83	43.18
V	0.27±0.09	0.50	0.13	32.88
Cr	1.53±0.67	3.31	0.70	43.51
Co	0.16±0.09	0.41	0.06	52.59
Ni	2.52±1.53	6.13	0.69	60.83
Cu	2.60±0.90	4.64	1.28	34.79
Zn	23.21±9.84	45.79	9.09	42.39
As	0.26±0.08	0.44	0.14	29.81
Se	0.08±0.04	0.22	0.04	46.74
Mo	0.04±0.03	0.12	0.01	64.43
Cd	0.15±0.07	0.30	0.03	44.84
Pb	3.04±2.02	9.55	1.00	66.24
Sb	0.02±0.01	0.07	0.00	66.95

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Keywords: *Parmotrema tinctorum*, inductively coupled plasma mass spectrometry (ICP-MS), wet digestion, dry ashing, ultrasound-assisted extraction