

Authenticating Geographical Origin of Tea Using ICP-MS and Agilent Mass Profiler Professional Software

Elemental profiling of Indian tea using Agilent 7850 ICP-MS and MPP chemometrics software



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Introduction

Tea is one of the most popular and widely consumed beverages in the world, making it a highly valuable agricultural commodity (1). India is the world's second largest producer of tea behind China, with most production based in the north-eastern states of Assam, West Bengal, and Tripura. Other tea plantations are in Kangra (North India) and Nilgiri (South India). Overall, Indian tea is categorized into three types: Assam (most grown variety), Darjeeling (superior quality and highest market value), and Nilgiri (known for its subtle flavors) (2).

Manufacturers blend different varieties of tea to create consistent products with a well-balanced flavor. Also, blending helps to take the pressure off high-demand tea varieties such as Darjeeling that is only grown in West Bengal. However, if a consumer buys a packet of Darjeeling tea, it is difficult for them to know the quality—and origin—of the tea based on the labeling. Increasingly, tea varieties with a high market value such as Darjeeling are a target for food fraudsters, who routinely substitute, adulterate, or mislabel food items for financial gain (3). Teas (and other food products) that are promoted based on their region of origin are more vulnerable to adulteration, especially when demand for the product is higher than the amount that can be produced. To protect the interests of consumers and producers from food fraud, analytical methods are needed to distinguish the geographical origin of tea and verify its authenticity.

Plant variety, soil type, environmental factors, and agricultural practices including irrigation and use of fertilizers are factors that affect the elemental composition of agricultural products used to produce foods and beverages. The analysis of a wide range of elements in foods is often performed using ICP-MS, following microwave-assisted acid digestion of samples (4–6). The multi-element analytical results provide an “elemental fingerprint” of each sample that is representative of the sample’s geographic origin. This data is useful for authentication studies and has been applied to foods including tea, spices, and rice (3, 7, 8).

Agilent ICP-MS instruments use an Octopole Reaction System (ORS⁴) that is optimized for the removal of all common polyatomic overlaps using helium (He) collision mode and kinetic energy discrimination (KED) (9). He KED mode allows the same cell settings to be used for many elements, providing high-quality, multi-element data sets needed for food-authenticity studies. Agilent ICP-MS data is compatible with Agilent Mass Profiler Professional (MPP), a chemometrics software package that is designed to process large sets of mass spectrometry (MS) data. MPP includes a choice of data classification methods, enabling analysts to build robust models to predict attributes such as sample origin of unknown samples.

Agilent ICP-MS, such as the 7850 and 7900, have a linear-dynamic-range over 10 or 11 orders of magnitude of concentration, enabling the measurement of major and trace analytes in samples in the same run. The wide dynamic range simplifies method setup by removing the need for custom-tuning conditions for major elements, while also ensuring that fewer reruns occur due to over-range results.

During this elemental profiling study, 150 tea samples were analyzed using an Agilent 7850 ICP-MS. The samples were collected in the same growing season from eight regions of India. Elemental data obtained from the 7850 ICP-MS was processed using MPP software. An initial Principal Component Analysis (PCA) showed that the geographical origin of tea could be differentiated based on differences in the concentrations of 18 of the 68 elements that were measured. So these 18 elements were selected for the subsequent analysis. The MPP software was used to build prediction models using different class prediction algorithms, and the prediction models were used to identify the origins of 24 ‘unknown’ test samples.

Experimental

Calibration standards

Calibration standards for the 18 “indicator” elements were prepared in 5% HNO₃ by volume (v/v). Calibration standards were prepared from Agilent standard solutions including multi-element calibration standard-1 (p/n 8500-6944), multi-element calibration standard-2A (p/n 8500-6940), environmental spike mix (p/n 5183-4687), and multi-element calibration standard-4 (p/n 8500-6942). Twelve elements (B, V, Cr, Co, Ni, Zn, Se, Rb, Sr, Mo, Cs, and Ba) were calibrated from 0.1 to 200 ppb; Mg from 1.0 to 20 ppm; Mn and Al from 0.1 to 2.0 ppm; La, Ce, and Nd from 0.1 to 20.0 ppb. The rhodium (Rh) internal standard (ISTD) solution was prepared from an Agilent single element Rh standard (p/n 5190-8509). The ISTD solution (1.00 mg/L) was mixed with the sample online using a tee connector.

Sample preparation

A total of 150 samples of tea from eight regions (Dooars, Cachar, Terai, Darjeeling, Tocklai, Tripura, Upper Assam, and North Bank) were provided by the Tea Research Association, India. Each sample was dried and ground, and about 0.50 ± 0.01 g of the sample was accurately weighed into a polytetrafluoroethylene (PTFE) microwave digestion vessel. Each sample was predigested in 5 mL HNO₃ and 0.1 mL HCl before applying the microwave digestion program (Anton Paar, Austria) shown in Table 1. Once cooled to room temperature, the digested solutions were transferred to 50 mL centrifuge tubes and made up to 40 mL using de-ionized water. All the samples, method blanks, and spiked samples were prepared using the same procedure (Figure 1).

Table 1. Microwave digestion program.

Step	Ramp Time (minutes)	Temperature, °C	Hold Time (minutes)
I	15	105	3
II	10	160	3
III	10	190	10
IV	-	Ambient temperature	-

Spiked sample preparation

To test the accuracy of the quantitative method, a spike recovery study was carried out by spiking a randomly selected sample from the North Bank region with elements of known concentrations. Of the elements analyzed, tea samples are generally rich in Zn, Al, Mg, and Mn, contain moderate concentrations of B, V, Cr, Co, Ni, Rb, Sr, Mo, and Sr, and lower levels of Se, Cs, La, Ce, and Nd. Therefore, before digestion, the tea sample from the North Bank region was spiked with these elements at 1 ppm, 100 ppb, and 10 ppb, respectively, relative to the final sample digest solution. The selected North Bank tea was spiked and prepared in triplicate.

Instrumentation

The Agilent 7850 ICP-MS, which includes the Ultra High Matrix Introduction (UHMI) system and ORS⁴ collision/reaction cell, was used for the analysis. Sample introduction was performed using an Agilent SPS 4 autosampler. The 7850 was configured with the standard configuration consisting of a MicroMist nebulizer, quartz spray chamber, 2.5 mm injector quartz torch, off-axis ion lens assembly, and nickel cones. All elements were measured using one set of He KED collision cell mode conditions, as detailed in Table 2. The 7850 was controlled using Agilent ICP-MS MassHunter software and the data was processed using MPP software (version 15.1).

Table 2. Agilent 7850 ICP-MS operating conditions.

Parameters	Value
RF Power (W)	1550
Sampling Depth (mm)	9.0
Nebulizer Gas Flow (L/min)	0.92
Spray Chamber Temperature (°C)	2.0
Lens Tune	Autotune
He Flow Rate (mL/min)	4.3
KED (V)	3

Results and discussion

Detection limits

During method development, data was collected for 68 elements. However, only 18 elements showed enough variation to be used in the class-prediction modeling. Instrument detection limits (IDLs) for the 18 elements were calculated as 3 x standard deviation (SD) of the concentration of the calibration curve blanks (n=10). Method detection limits (MDLs) were calculated after applying the dilution factor of 80 (0.50 ± 0.01 g of tea digested and diluted to a final volume of 40 mL). The IDLs and MDLs are shown in Table 3. The low IDLs demonstrate the suitability of the 7850 ICP-MS for the determination of trace elements.

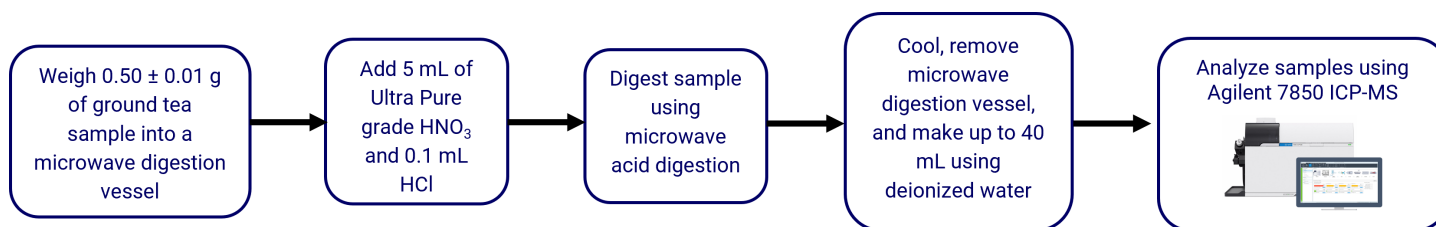


Figure 1. Analytical workflow used for the analysis of tea by ICP-MS.

Table 3. Calibration coefficient (r), IDLs, and MDLs of the 18 elements used to distinguish the geographical origin of tea.

Element	R Value	IDL, µg/kg	MDL, µg/kg
11 B	0.9999	0.069	5.48
24 Mg	0.9999	0.003	0.24
27 Al	0.9997	0.007	0.55
51 V	0.9999	0.0003	0.02
52 Cr	0.9999	0.0002	0.01
55 Mn	0.9999	0.017	1.35
59 Co	0.9999	0.003	0.24
60 Ni	0.9997	0.0004	0.03
66 Zn	0.9999	0.001	0.10
82 Se	0.9999	0.004	0.32
85 Rb	1.0000	0.0003	0.02
88 Sr	1.0000	0.0002	0.01
95 Mo	1.0000	0.0003	0.02
133 Cs	0.9999	0.0001	0.01
137 Ba	1.0000	0.0005	0.04
139 La	0.9997	0.00005	0.004
140 Ce	0.9997	0.002	0.13
146 Nd	1.0000	0.001	0.08

Internal standard stability

Rh was used as the ISTD as it was not expected to be present in the tea samples, unlike other potential ISTD elements such as Li and Sc. The analytical sequence comprising 150 tea sample digests, method blanks, quality control (QC) checks, and spike recovery solutions (280 runs in total) was analyzed repeatedly over 11 hours. As shown in Figure 2, all the ISTD recovery measurements were within the $\pm 20\%$ limits (indicated by the red dotted line). The recovery test shows that the plasma of the 7850 was able to decompose the variable sample matrices effectively, enabling excellent stability to be maintained over the long run. Also, no significant matrix deposition occurred on the interface during the sequence.

Quality control check

As a QC check, standards containing 10.0 µg/L of 15 elements and 1.0 µg/L of La, Ce, and Nd were analyzed before and after the batch analysis. The drift (% difference) between the measurements was less than 5.0% for all the elements, again demonstrating the stability and robustness of the 7850 ICP-MS (Table 4).

Table 4. QC check solution measured before and after the 11-hour run using the Agilent 7850 ICP-MS.

Element	Concentration in QC Solution µg/L	Measured Concentration		
		Run 1, µg/L	Run 2, µg/L	*Drift (%)
11 B	10.0	9.94	9.70	2.5
24 Mg	10.0	10.37	9.87	5.0
27 Al	10.0	10.70	10.46	2.4
51 V	10.0	9.93	9.61	3.2
52 Cr	10.0	9.87	9.55	3.1
55 Mn	10.0	9.82	9.44	3.8
59 Co	10.0	9.88	9.66	2.2
60 Ni	10.0	9.59	9.33	2.6
66 Zn	10.0	10.07	9.91	1.6
82 Se	10.0	10.53	10.04	4.9
85 Rb	10.0	9.69	9.36	3.2
88 Sr	10.0	9.67	9.35	3.2
95 Mo	10.0	9.60	9.67	0.7
133 Cs	10.0	9.58	9.41	1.8
137 Ba	10.0	9.97	9.93	0.4
139 La	1.0	0.95	0.95	0.1
140 Ce	1.0	0.95	0.95	0.0
146 Nd	1.0	1.00	1.00	0.0

*Difference in concentration of QC check solution measured before batch analysis (run 1) and after batch analysis (run 2).

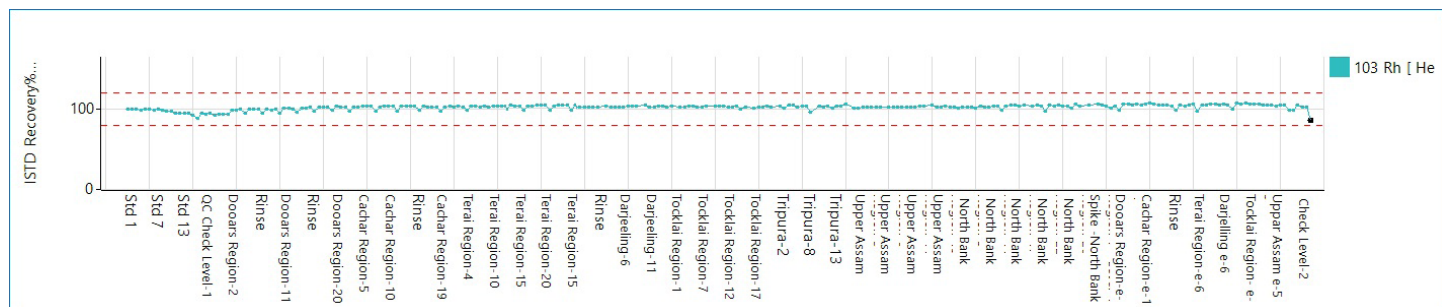


Figure 2. Recovery of ISTD measured over 11 hours (280 analyses) using the Agilent 7850 ICP-MS. Due to limited space, not all sample names are shown.

Quantitative results for tea samples

Tea digests from eight different regions of India were analyzed using the 7850 ICP-MS. As shown in Table 5, the % variation of the quantitative results was <5% for each region.

Table 5. Mean measured concentrations of 18 elements in 15 or 20 tea samples from eight regions, corrected for dilution. Units: mg/kg.

Element	Dooars Region		Cachar Region		Terai Region		Darjeeling Region		Tocklai Region		Tripura		Upper Assam		North Bank	
	Average Conc (n=20)	Variation (%)	Average Conc (n=20)	Variation (%)	Average Conc (n=20)	Variation (%)	Average Conc (n=15)	Variation (%)	Average Conc (n=20)	Variation (%)	Average Conc (n=15)	Variation (%)	Average Conc (n=20)	Variation (%)	Average Conc (n=20)	Variation (%)
B	12	2.0	15	1.8	9.7	2.1	12	2.1	6.4	2.3	14	3.2	9.9	2.4	11	2.7
Mg	2245	0.6	1989	0.9	2183	1.3	1951	0.8	2337	1.5	1418	2.9	2162	1.5	1988	2.2
Al	853	0.7	734	0.8	922	1.3	559	1.2	579	1.5	349	3.0	630	1.5	759	2.0
V	0.19	4.9	0.22	2.8	0.25	3.6	0.25	2.7	0.23	2.4	<MDL	-	0.10	3.6	8.3	3.0
Cr	7.9	4.6	19	1.0	21	1.1	0.54	2.4	7.9	1.6	0.32	4.9	0.84	2.1	12	2.7
Mn	639	0.8	637	1.0	504	1.2	384	1.0	817	1.3	540	3.0	308	1.3	1088	2.0
Co	0.18	4.4	0.34	1.6	0.39	2.8	0.14	4.2	0.30	1.3	0.15	2.9	0.12	2.6	1.2	2.8
Ni	5.9	4.3	7.7	1.1	7.6	2.7	8.2	0.9	8.0	1.7	3.4	2.8	2.5	1.3	8.9	3.8
Zn	32	4.4	20	0.9	33	4.9	41	0.9	37	1.5	27	2.9	27	1.6	31	2.2
Se	0.85	3.9	0.27	4.9	0.19	4.8	0.19	4.7	<MDL	-	0.20	4.1	0.31	4.6	0.19	3.1
Rb	23	0.5	44	1.0	67	1.6	71	1.4	29	1.4	54	2.9	35	1.5	93	2.2
Sr	6.3	0.7	11	1.2	12	1.6	12	1.0	16	1.3	13	2.9	16	1.5	11	2.3
Mo	0.90	2.9	0.25	3.1	0.23	3.6	<MDL	-	<MDL	-	<MDL	-	<MDL	-	1.0	4.8
Cs	0.36	2.9	0.16	1.6	0.17	1.7	0.10	2.0	<MDL	-	0.12	2.9	0.13	2.1	0.13	2.0
Ba	19	4.9	31	1.0	33	0.8	21	0.9	33	1.2	31	2.9	37	1.4	41	2.2
La	0.13	1.7	0.40	1.1	0.15	2.0	0.16	1.2	0.14	1.5	0.12	2.9	0.15	1.9	0.21	4.3
Ce	0.21	1.1	0.33	1.0	0.28	1.8	0.28	1.5	0.24	2.5	0.18	3.0	0.20	1.4	0.38	2.2
Nd	0.10	2.4	0.29	2.2	0.12	3.1	0.14	2.2	0.12	3.8	<MDL	-	0.21	1.7	0.24	3.0

Spike recoveries and evaluation of matrix effects

To test the accuracy of the quantitative method, a spike recovery study was carried out on one of the tea samples from the North Bank region. Each of the three different spiked samples of the tea sample was analyzed and the spike recoveries were calculated, as shown in Table 6. The average of the spike recoveries (n=3) was within 100 ± 10% with %RSD of the measurements less than 5%. The excellent recoveries indicate that all elements were unaffected by any matrix effects arising from the sample matrix.

Table 6. Accuracy and precision of analysis of a North Bank spiked tea sample prepared in triplicate by Agilent 7850 ICP-MS.

Element	Concentration of Unspiked Sample, mg/kg	Spike Concentration, mg/kg	Concentration of Spiked Sample, mg/kg	Average Recovery, % (n=3)	RSD, %
11 B	11.4	8	18.7	92	3.7
24 Mg	1987	80	2071	105	4.8
27 Al	763	80	837	93	4.0
51 V	8.3	8	16.5	103	0.7
52 Cr	12.0	8	20.6	107	1.1
55 Mn	1087	80	1160	92	2.1
59 Co	1.4	8	9.5	101	2.0
60 Ni	8.7	8	16.8	102	1.0
66 Zn	33	80	118	107	1.4
82 Se	0.18	0.8	0.99	101	1.4
85 Rb	94	8	101	97	1.7
88 Sr	11.5	8	18.9	93	3.3
95 Mo	0.98	8	8.7	96	0.5
133 Cs	0.14	0.8	0.87	92	0.8
137 Ba	42	8	49	95	2.2
139 La	0.23	0.8	0.99	95	3.6
140 Ce	0.39	0.8	1.21	103	4.0
146 Nd	0.25	0.8	1.1	101	3.5

Data analysis using MPP software

The ICP-MS data for 18 elements measured in 150 tea samples were combined and imported into Agilent MPP chemometric software for statistical analysis. The MPP software includes various tests such as t-test, analysis of variance (ANOVA), model building algorithms, BoxWhisker, correlation and clustering analysis.

Principal Component Analysis

A PCA (with a P cut-off value <0.05) was performed to see if there were significant differences between tea sample groups based on geographical region. The PCA technique evaluates the relative contribution of elements to the separation of the groups. The three dimensional (3D)-PCA scores in Figure 3 show significant variation between tea samples from different geographical origin based on elemental profiling of 18 elements. A total of 72% of the variance ratio was captured in first three dimensions PC1 (X-axis, 38.68%), PC2 (Y-axis, 19.87%), and PC3 (Z-axis, 13.66%). According to the PCA loading values, the separation between the geographical origin of tea was driven by Sr, Ba, and B for PC1; Cs, La and Rb for PC2; and Mo, Ce, and Nd for PC3.

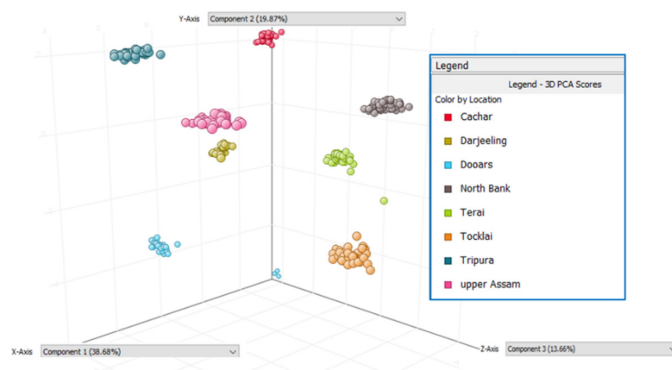


Figure 3. 3D-PCA plot scores of 150 tea samples showing the discrimination in geographical origin based on elemental profiling. The X-axis, Y-axis, and Z-axis of the plot represent the three major principal components of the PCA results.

Class prediction analysis

Class prediction analysis is a useful technique that enables unknown samples to be assigned to a previously determined group in an unbiased fashion. The MPP software includes several class prediction algorithms. The choice of algorithms includes soft independent modeling of class analogy (SIMCA), random forest, linear discriminant analysis (LDA), partial least squares discriminant analysis (PLSDA), decision tree, support vector machine (SVM), naive Bayes, and neural network. A classification model can be built using any of these class prediction algorithms.

Building a prediction model

LDA and SVM, which are commonly used class prediction algorithms for foods, were used to build prediction models for identifying the geographical origin of tea samples. All the interpretations were carried out using non-averaged location values. To build the models, tea samples were selected at random from each of the eight regions (12 samples from Darjeeling and Tripura, and 18 from all other regions). The remaining 24 samples (three from each of the eight regions) were used as unknowns to test the accuracy of both prediction models.

Testing the prediction model

The predicted location results obtained using the LDA and SVM models are shown in Table 7. The locations of all 24 'unknown' tea samples were correctly identified using both prediction models, with high degrees of confidence.

Table 7. Results of the predicted location results for the 24 'unknown' tea samples. Values close to 1 indicate a high degree of confidence.

Sample Name	Actual Location	SVM Prediction Model		LDA Prediction Model	
		Predicted Location	Confidence Measure	Predicted Location	Confidence Measure
Unknown C1	Cachar	Cachar	0.907	Cachar	0.986
Unknown C2	Cachar	Cachar	0.910	Cachar	0.967
Unknown C3	Cachar	Cachar	0.907	Cachar	0.977
Unknown D1	Dooars	Dooars	0.912	Dooars	0.976
Unknown D2	Dooars	Dooars	0.916	Dooars	0.966
Unknown D3	Dooars	Dooars	0.905	Dooars	0.964
Unknown Da1	Darjeeling	Darjeeling	0.886	Darjeeling	0.981
Unknown Da2	Darjeeling	Darjeeling	0.891	Darjeeling	0.979
Unknown Da3	Darjeeling	Darjeeling	0.906	Darjeeling	0.980
Unknown NB1	North Bank	North Bank	0.919	North Bank	0.978
Unknown NB2	North Bank	North Bank	0.921	North Bank	0.989
Unknown NB3	North Bank	North Bank	0.919	North Bank	0.989
Unknown T1	Tripura	Tripura	0.898	Tripura	0.985
Unknown T2	Tripura	Tripura	0.886	Tripura	0.977
Unknown T3	Tripura	Tripura	0.877	Tripura	0.940
Unknown Te1	Terai	Terai	0.902	Terai	0.980
Unknown Te2	Terai	Terai	0.905	Terai	0.986
Unknown Te3	Terai	Terai	0.911	Terai	0.983
Unknown To1	Tocklai	Tocklai	0.884	Tocklai	0.987
Unknown To2	Tocklai	Tocklai	0.887	Tocklai	0.981
Unknown To3	Tocklai	Tocklai	0.895	Tocklai	0.993
Unknown UA1	Upper Assam	Upper Assam	0.901	Upper Assam	0.985
Unknown UA2	Upper Assam	Upper Assam	0.913	Upper Assam	0.988
Unknown UA3	Upper Assam	Upper Assam	0.910	Upper Assam	0.985

Conclusion

Sixty eight major and trace elements were measured in 150 tea samples from eight regions of India using the Agilent 7850 ICP-MS. The wide dynamic range and helium collision cell mode of the 7850 enabled all elements to be measured in a single batch file using the same operating conditions. The multielement data batch was processed using Agilent Mass Profiler Professional (MPP) chemometrics software. Applying Principal Component Analysis (PCA) to the data showed some variation in the concentrations of 18 elements in tea according to region. Two class prediction models (SVM and LDA) that are often used in food-authentication studies were used to build class prediction models based on the 18 elements. Both models correctly identified the geographical origin of 24 'unknown' tea test-samples, including high-value Darjeeling.

The elemental fingerprint ICP-MS method has the potential to characterize the geographical origin of a wide range of foodstuffs. The results can be used to confirm the origin of authentic products or identify products that have been adulterated or mislabeled.

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