Direct Analysis of 13 Trace Elements in Tea Infusions using ICP-MS with Integrated Sample Introduction System in Discrete Sampling (ISIS-DS)

Application Note

Food Testing and Agriculture

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Introduction

Tea, when prepared by infusion of leaves, flowers, or roots, has generated significant scientific interest due to its increased consumption, antioxidant activity, and presence of some micronutrients, such as minerals, flavonoids, and catechins [1]. Studies have shown a presence of potentially toxic and cumulative substances in herbs such as inorganic contaminants [2]. These contaminants originate from different aspects of the herbal tea manufacturing process and include sources such as soil and water, fertilizers, and airborne industrial emissions [3]. Depending on the concentration, potentially toxic elements can cause damage to human health ranging from liver and kidney dysfunctions to carcinogenesis. However, not all contaminants remain in the tea infusion. Most residual toxins are present in very low levels, lowering the risk associated with tea ingestion.

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One of the most common methods to assess trace multi-element concentrations in tea infusion samples is ICP-MS elemental determination after sample digestion. This method requires microwave digestion systems and reagents such as concentrated acids and oxidant agents. The whole process can achieve dilution factors of 10–100 times. This approach requires long run times and excess reagents, often resulting in poor method detection limits. Conversely, direct analysis also compromises performance parameters, such as precision, long-term stability, residue build-up, and increased maintenance.

The Agilent Integrated Sample Introduction System (ISIS) accessory for 7700x/7800 ICP-MS incorporates the Discrete Sampling (DS) mode for direct analysis. This provides the advantage of accurate sample use, avoiding unnecessary sample exposure by the ICP-MS sample introduction system. In addition, DS mode has the ability to maintain instrument performance and provide high speed analysis when combined with the unique capabilities of ORS[3] and He as collision gas.

The synergy between a collision cell that operates effectively with an inert cell gas (helium) and discrete sampling results in excellent performance as measured by detection limits, precision, and throughput. This application note demonstrates the benefits of combining helium collision mode and discrete sampling to develop and validate an easy-to-use, polyatomic interference-free method for the determination of 13 elements in tea infusion.

Experimental

The Agilent 7700x ICP-MS System was fitted with an Agilent I-AS Autosampler with a standard sample introduction system, which includes a MicroMist glass concentric nebulizer, quartz double pass spray chamber, connector tube with dilution port, a quartz torch with a 2.5-mm id and standard nickel cones. Discrete Sampling (DS) is performed with the ISIS accessory set up with one peristaltic pump and one 6-port switching valve. It is coupled to the ICP-MS to deliver only the required amount of sample, achieving productivity gains, and further improving long-term matrix tolerance while reducing run time and sample exposure as much as possible. A preconfigured ISIS tubing kit for ISIS-DS is available for easy setup and convenience.

Figure 1 shows a schematic of how the ISIS-DS system works. While there is a continuous flow of the internal standards and carrier solutions, the sample fills the loop with the 6-port valve in the load position. The valve then switches to the injection position, sending the carrier solution through the sample loop and pushing the sample into the nebulizer.

A detailed description of the ISIS-DS operation can be found in previous publications [4,5].

The 7700x tuning was based on a preset method for food and clinical applications using ICP-MS MassHunter software. No Gas, He, and HEHe modes were used in this method and the tuning conditions can be found in Table 1.

For calibration, all 13 elements were divided into two groups; one in the lower range (0, 0.1, 0.5, 1, 5, 10, and 100 µg/L) including As, Cd, Cr, Cu, Hg, Ni, Pb, and Se, and the other in the higher range (0, 10, 100, 250, 500, 1,000, and 2,000 µg/L) including Al, Ba, Fe, Mn, and Zn. Both analytical curves were made in HNO_3 0.2% v/v. The method did not require matrix matching or recalibrations. A Sc, Ge, and Y solution containing 250 µg/L of each element was used as online internal standard.

After initial calibration, a sequence with 10 analytical blanks was run to find standard deviation (s) and calculate detection limits (DL = 3 × s) and quantification limits (QL = 10 × s). Repeatability was calculated using coefficient of variation (CV with n = 16). Due to the lack of certified reference materials for this matrix, accuracy was assessed through recovery of the analytes after spikes. In both cases (precision and accuracy), the infusion samples were spiked at different concentration levels: As, Cd, Cr, Cu, Hg, Ni, Pb, and Se at 10 and 50 µg/L; Al, Ba, Fe, Mn, and Zn at 250 and 500 µg/L.
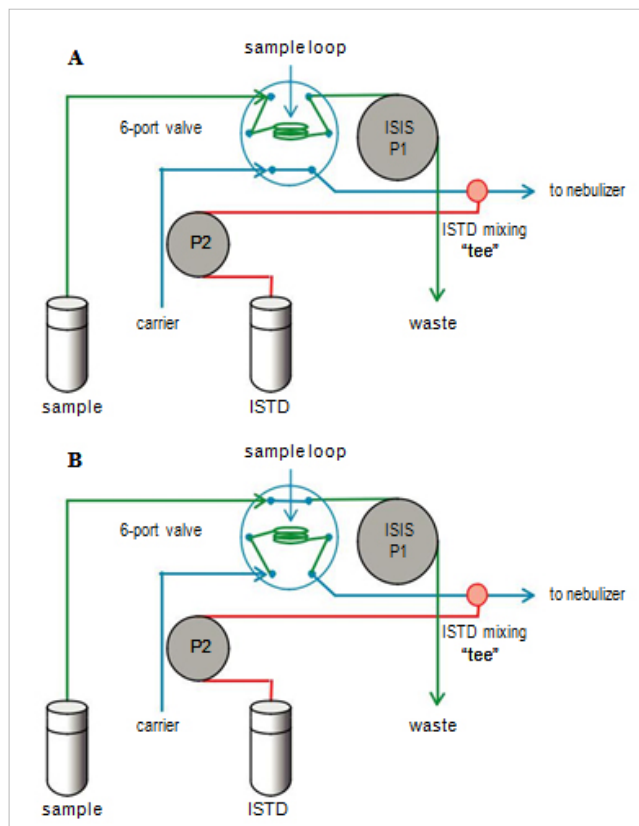
Figure 1. ISIS operation at DS mode: Valve in load position (A) and injection position (B.

Table 1. Agilent 7700x ICP-MS and ISIS-DS Operating Conditions

Agilent 7700x parameters	
RF power	1,550 W
Carrier gas flow	1.1 L/min
Number of elements/internal standards	13/3
Replicate/peak pattern/sweeps	3/3/100
Reading time	60 seconds
Cell tuning modes	No Gas/ He/ HEHe
Shift time between modes(No Gas/He/HEHe)	5 seconds
He cell flow on HEHe mode	10 mL/min
Kinetic energy discrimination on HEHe mode	7 V
He cell flow on He mode	5 mL/min
Kinetic energy discrimination on He mode	5 V
Agilent ISIS-DS parameters	
Loop volume and size	150/30 (µL/cm)
Uptake time	20 seconds
Acquisition delay	20 seconds
Rinse time	Pre-emptive rinse during acquisition

Method performance was verified using four varieties of tea as detailed in Table 2. For each variety, the study tested three different brands found in a local market (2013 Campinas, SP - Brazil), and for each brand, three infusions were prepared. Infusion preparation was done by boiling 200 mL of deionized water (18.2 MW.cm) with 1.5 g of sample (equivalent to a commercial tea bag) for 3 minutes. After cooling, the infu-sions were acidified (HNO_3 0.2% v/v) and filtered through a 250-µm polymeric membrane and transferred to the autosampler vials.

**Table 2.** Tea Samples Used in Method Application

Lemon balm	Leaves of Melissa officinalis, L.
Fennel	Fruits of Pimpinella anisum, L.
Flowers and fruits	Apple Fruit - *Pyrus malus L.* Flowers Hibiscus - *Hibiscus sabdariffa, L.* Fruits and Flowers Wild Rose - *Rosa canina, L.* Orange Peel - *Citrus aurantium, L.* Lemon Peel - *Citrus Limonum, (L.)* Osbeck leaves Stevia - *Stevia rebaundiana, Bert*
Red	Stems and leaves of Camellia sinensis (L.) Kuntze

Results and Discussion

Routine methods for this application require high througput, low DLs, and low CV. All these figures of merit are discussed below.

Productivity and stability

In an analysis process, one of the most time-consuming steps is sample preparation. The replacement of conventional digestion by direct sample introduction dramatically reduces the analysis time. Some standard features of the Agilent 7700/7800 ICP-MS such as the MicroMist nebulizer, Peltier-cooled double pass spray chamber, High Matrix Introduction (HMI) system, wide diameter (2.5-mm id) torch injector, and solid state RF (27 MHz) generator are important to ensure a robust performance in challenging matrices such as those used in this study. These features result in a very robust plasma with CeO^+/Ce^+ ratios lower than 1.5%. Even with this robust plasma performance, a long routine with direct tea sample injections can cause cone buildup and internal standard instability. This requires maintenance stops for cleaning or replacement of some sample introduction parts. With ISIS-DS, it is possible to overcome these problems by reducing ICP-MS sample exposure by 3 to 4 times, resulting in increased overall system stability. It is possible to have an estimate of the system stability from the 2-hour internal standard profile run shown in Figure 2.
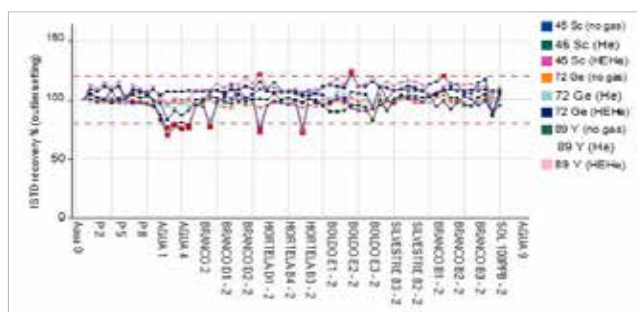
Figure 2. Internal standard recoveries normalized to the calibration blank for all samples.

Comparing total analysis time per sample (sample uptake, stabilization, reading, and rinse times), DS-ICP-MS achieved a 30% cycle time reduction over conventional ICP-MS (197 seconds to 136 seconds). This analysis time reduction was achieved by ISIS at DS mode only. The ORS^3 contribution must also be considered, because it can resolve almost every polyatomic interference using just He in different modes (regular He mode and HEHe mode), and its small volume allows the shift between No Gas, He, and HEHe modes in approximately 5 seconds. The analyst can then choose more than one cell mode in the same method with no significant increase in analysis time.

Regarding the food quality control, some elements can be toxic contaminants such as Al, As, Ba, Cd, Hg, Ni, and Pb; nutrients such as Fe, Mn, and Zn; or even both such as Cu, Cr, and Se. Usually, the contaminants are present at low levels in a sample, while nutrients can be present at higher levels. This is a challenge faced by food analysts, because it is difficult to combine different concentration ranges in a single method or even in a single instrument. The Agilent 7700/7800 ICP-MS dual stage detector can obtain calibration curves for all these elements in different ranges with linearity (R^2) greater than 0.9999.

Detection limits, precision, and recovery

Direct analysis of tea samples minimized sample handling, thereby decreasing the risk of contamination and hazardous situations with chemical handling. In addition, this method does not require dilution, thereby improving detection limits. The fast shift between cell modes provides the opportunity for the user to analyze with three different tune modes taking the best of each one. For example, $^{52}Cr^+$, which has a strong interference from $^{12}C^{40}Ar^+$, but in this application Cr could be easily quantified in He mode, while $^{202}Hg^+$, which has no polyatomic interference observed in tea infusion analysis could be analyzed in No Gas mode. This feature lowers detection limits. Another challenge in lowering detection limits is the potential for contamination in noncontrolled environments. This affects elements such as Al, Cu, Mn, and Zn. Table 3 lists DLs, QLs, and other performance measures as well as the best analysis conditions for the analytes studied.

Using the conditions proposed by this method, it was possible to keep the precision values less than 20%, and recoveries in the two levels between 82 and 121%

Table 3. Analysis conditions and performance measures

Iso-tope	Cell	ISTD	Limits (µg/L) DL (3s)	Limits (µg/L) QL (10s)	Precision (%)	Recovery (%) Level 1	Recovery (%) Level 2
^{27}Al	He	^{72}Ge	6	20	13	103 ± 5	99 ± 3
^{52}Cr	He	-	0.09	0.29	17	85 ± 1	91 ± 1
^{55}Mn	He	^{72}Ge	0.11	0.38	11	87 ± 4	89 ± 6
^{56}Fe	HEHe	^{72}Ge	0.66	2.2	8	85 ± 4	92 ± 3
^{60}Ni	He	-	0.63	2.1	10	82 ± 1	89 ± 1
^{63}Cu	-	^{72}Ge	0.51	1.7	2	95 ± 5	92 ± 1
^{66}Zn	He	^{72}Ge	10	35	15	95 ± 6	95 ± 3
^{75}As	HEHe	^{89}Y	0.14	0.46	4	96 ± 2	104 ± 2
^{80}Se	HEHe	-	0.15	0.50	3	106 ± 2	110.5 ± 0.4
^{111}Cd	-	^{72}Ge	0.016	0.053	4	99 ± 1	100 ± 1
^{138}Ba	He	^{72}Ge	0.60	2.0	12	110 ± 8	111 ± 3
^{202}Hg	-	^{45}Sc	0.10	0.34	9	111 ± 4	121.0 ± 0.4
^{206}Pb	HEHe	^{72}Ge	0.12	0.39	6	112 ± 3	113 ± 2

Sample analysis

After optimizing analysis conditions and evaluating the method performance, three samples of four selected varieties of tea were analyzed in triplicate to verify the performance of the method while running real samples. Table 4 shows the results.

The data show the method capability, using the discrete sampling system ISIS-DS (without sample dilution), for quantifying extremely low concentrations of several elements, including those that present risk to human health (such as As, Cd, Cr, Hg, and Pb).

Results showed a large variation in concentration values when comparing different varieties of tea infusions. It was determined that the infusion of red tea had the highest concentrations of Al, As, Cr, Mn, Ni, and Pb. However, no sample presented values higher than the maximum established in Resolution 12/11 where the stated limits are: 600 µg As/L, 600 µg Pb/L, and 400 µg Cd/L [7].

Table 4. Average Result and Concentration Range of the Tea Infusions Analyzed

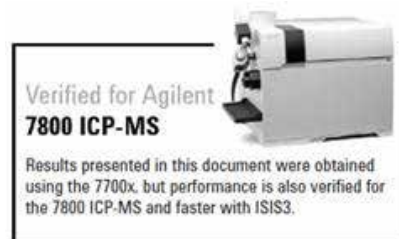|		Average result and range (n = 3, µg/L)		
Element	Lemon balm	Fennel	Flowers and fruits	Red
Al	ND < 20	32 (31–34)	28 (24–32)	954 (882–1,033)
As	ND < 0.46	ND < 0.46	0.18 (ND < 0.46–0.53)	0.76 (ND < 0.46–1.8)
Ba	16 (8.2–21)	7.7 (7.0–8.1)	103 (89–111)	15(13 - 20)
Cd	0.093 (ND < 0.053–0.21)	ND < 0.053	0.021 (ND < 0.053–0.063)	ND < 0.053
Cr	ND < 0.29	ND < 0.29	0.33 (ND < 0.29–0.53)	0.66 (0.58–0.74)
Cu	7.7 (5.2–9.2)	21 (19–22)	9.8 (6.1–13)	6.3 (4.5–9.7)
Fe	18 (11–24)	53 (27–70)	91 (70–102)	65(47–81)
Hg	ND < 0.34	ND < 0.34	ND < 0.34	ND < 0.34
Mn	509 (157–896)	56 (47–62)	405 (357–434)	1020 (951–1,131)
Ni	ND < 2.1	ND < 2.1	3.2 (2.1–4.5)	19(16–22)
Pb	0.43 (ND < 0.39–0.86)	ND < 0.39	0.22 (ND < 0.39–0.65)	0.78 (ND < 0.39–1.3)
Se	ND < 0.50	ND < 0.50	ND < 0.50	ND < 0.50
Zn	21 (ND < 35–62)	42 (37–46)	ND < 35	30(ND < 35 - 45)

*ND = Non Detected

Conclusion

This application note describes the development and validation of an analytical methodology for the determination of the total concentration of 13 potentially toxins and micronutrients in tea infusions using a direct analysis approach with ISIS-DS. This method provides significant benefits in terms of reduced cycle time and sample handling. Trace amounts for all 13 chemicals were found to be below published regulatory limits [7].

This method can be used to monitor tea infusions with good precision and accuracy and can also be adapted for other drinks such as coffee and fruit juices.

References

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Results presented in this document were obtained using the 7700x, but performance is also verified for the 7800 ICP-MS and faster with ISIS3.

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