

Determination of trace heavy metals in spices using single quadrupole ICP-MS

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Goal

To assess the suitability of Thermo Scientific™ iCAP™ RQ ICP-MS for the determination of trace elements in spices such as turmeric, black pepper, and chilli powder. To assess the method performance against the requirements of the AOAC 2015.01 guideline, the Food Safety and Standards Authority of India (FSSAI) and the China Food and Drug Administration (CFDA).

Introduction

Spices or condiments are usually dried parts of plants, such as fruits, seeds, barks, flowers, roots, and rhizomes. Typical spices such as turmeric, cinnamon, black pepper, and chilli powder have been used for thousands of years across the globe due to their culinary, anti-microbial, and presumed medicinal properties. The spice trade redrew the world map and came to define our global economy with a significant increase in spice trading observed across recent years.

Typically, spices are natural products grown in farmlands or harvested from forests. For spices originating from farms there are risks of contamination with heavy metals or other toxic elements, mainly due to the use of contaminated soil and water, administration of agrochemicals for pest control, milling, handling, and storage in preparation for commercialization. From the perspective of food safety,

contamination with such elements can lead to adverse effects on human health especially when present above certain thresholds. Hence, it is critical to continuously monitor and quantify the level of trace elements in food commodities including spices. FSSAI¹ and CFDA² provide regulations with recommended maximum residual limits (MRLs) in spices for heavy metals (Table 1).

Table 1. Target analytes with the FSSAI and CFDA MRL values in mg·kg⁻¹

Elements	FSSAI		CFDA	
	Spices	Turmeric powder	Spices	Turmeric powder
Mercury	1.0	1.0	-	-
Arsenic	5.0	0.1	-	-
Lead	10.0	10.0	3.0	-
Cadmium	1.5	0.1	-	-
Copper	30.0	5.0	-	-
Tin	250.0	Nil	250.0	-

* For all other elements in the scope of this study, no MRLs are available in the FSSAI and CFDA regulations.

Different analytical procedures have been reported in the literature for the determination of trace elements in spices, and inductively coupled plasma mass spectrometry (ICP-MS) is commonly accepted as the technique of choice^{3,4}. ICP-MS, predominantly with single quadrupole mass spectrometers, offers the advantage of high sensitivity, selectivity, wider dynamic range, and robust performance. In addition to the analytical system of choice, sample preparation is also important. Microwave-assisted acid digestion is the most common and preferred sample preparation technique for analysis by ICP-MS due to its advantages over the open digestion method⁵. When compared to the open digestion method, microwave digestion proficiently retains the volatile elements such as mercury in the sample solution, ensuring complete decomposition of the organic sample matrix at the same time.

Additionally, accurate and precise quantification of elemental impurities at trace level requires high sensitivity and efficient mechanisms for polyatomic interference removal. The Thermo Scientific iCAP RQ ICP-MS offers both higher sensitivity and effective interference removal by using kinetic energy discrimination (KED) acquisition mode in conjunction with pure helium in the collision reaction cell (CRC). The objective of this application note was to assess the suitability of the iCAP RQ ICP-MS for trace level quantification of various elements in spices using microwave-assisted digestion for sample preparation with reduced acid concentration.

Experimental

Chemicals and reagents

- Nitric acid (65–69%), TraceMetal™ Grade, Fisher Chemical™ (P/N A509-P212)
- Hydrogen peroxide (30–32%), TraceMetal™ Grade, Fisher Chemical™ (P/N H/1820/15)
- Hydrochloric acid (35–37%), TraceMetal™ Grade, Fisher Chemical™ (P/N A508-P500)
- Deionized water (18.20 MΩ·cm), Thermo Scientific™ Barnstead™ MicroPure™ Water Purification System
- Single element standard solutions (for all elements under study, each at 1000 mg·L⁻¹, Inorganic™ Ventures (Christiansburg, Virginia, USA)

Standard preparation and calibration

All the target elements considered for this experiment are shown in Table 4. Considering the MRLs and the expected concentrations of various elements present in the target matrices, four different levels of working standard mixtures were prepared separately in 4% nitric acid and 0.4% HCl (diluent) by using 1000 mg·L⁻¹ reference standards of individual analytes. Six calibration standard solutions were prepared by mixing appropriate volumes of different working solutions with the diluent mentioned above to achieve the expected linearity range. A solution containing the internal standards was added to each standard solution and blanks. Gold (200 µg·L⁻¹) was added to all solutions including blanks, standards, and rinse solutions to facilitate stability and wash out mercury to reduce memory effect.

Sample preparation

Quantification of multiple elements at low levels in rice samples was reported with sample preparation using a microwave digestion method,⁶ and a similar method was used here. Turmeric (*Curcuma longa*), black pepper (*Piper nigrum*), and red chilli (*Capsicum annuum*) powders were purchased from the local Ghaziabad market. The samples were homogenized and weighed accurately around 0.25 ± 0.025 g in a pre-cleaned, dry 75 mL volume microwave digestion vessel. For the accuracy study, samples were spiked with known analyte concentrations before the addition of any reagents. The required volume of gold standard stock solution was added to each vessel to maintain $200 \mu\text{g}\cdot\text{L}^{-1}$ of Au in the final solution. Additionally, 2 mL nitric acid (HNO_3), 1 mL hydrogen peroxide (H_2O_2), and 0.2 mL hydrochloric acid (HCl) were added. Vessels were allowed to stand in a fume hood to facilitate pre-digestion at atmospheric conditions for 15 min. The microwave digestion vessels were closed, and the microwave digestion process was initiated with a set temperature program given in Table 2 using a CEM MARS™ 6 microwave digestion system (CEM Corporation, Matthews, NC, USA).

After completion of the digestion method, the microwave digestion vessels were cooled at room temperature to cool the hot acid fumes and reduce the vapor pressure by keeping the rotor outside the system for 15 min. The vessels were opened slowly in a fume hood, as pressurized non-condensable gases could be released. The digested sample solutions were quantitatively transferred into 50 mL volumetric flasks pre-cleaned and rinsed multiple times with deionized water. The internal standards were added from a stock solution (containing $10 \text{ mg}\cdot\text{L}^{-1}$ of each element) to result in a final concentration of $20 \mu\text{g}\cdot\text{L}^{-1}$, and the total volume was made up to 50 mL with deionized water. The prepared sample solutions were vortexed well to ensure thorough mixing of the resulting solutions. The acid blank was prepared similarly by following the above procedure without a sample matrix.

Table 2. Temperature program for microwave digestion

	Ramp time	Hold time	Temperature	Power
Step 1	40 min	30 min	200 °C	1,500 W

Note: Ramp time and microwave power settings may vary depending on the number of vessels.

ICP-MS analysis

The iCAP RQ ICP-MS was set up to perform trace level measurement using a standard sample introduction system consisting of a glass concentric nebulizer, quartz cyclonic spray chamber, 2.5 mm quartz injector (P/N 1305600), and Ni-tipped sample and skimmer interface cones. The instrument was tuned using automatic tuning wizards available within Thermo Scientific™ Qtegra™ Intelligent Scientific Data Solutions (ISDS) Software to ensure expected performance.

To automate sample analysis, a Teledyne CETAC™ ASX 560 (Teledyne CETAC Technologies, Omaha, NE) autosampler was used in the experiment. A summary of the instrument parameters used in the study is presented in Table 3.

Table 3. iCAP-RQ ICP-MS parameters

Parameter	Value
Plasma power (RF)	1,550 W
Nebulizer gas	$1.06 \text{ L}\cdot\text{min}^{-1}$
Auxiliary gas	$0.8 \text{ L}\cdot\text{min}^{-1}$
Cool gas flow (Argon)	$14.0 \text{ L}\cdot\text{min}^{-1}$
CCT gas flow (He gas)	$5.1 \text{ mL}\cdot\text{min}^{-1}$
KED bias potential	3 V
Sample uptake/wash time	45
Dwell time	0.05 s
Number of readings per sample	3 Main runs with 10 sweeps each
Total acquisition time (3 repetitions including rinse)	150 s

Data acquisition and processing

Qtegra ISDS Software was used for data acquisition and processing. The process of method set up starting from the selection of the most appropriate isotopic mass for each analyte element, support of mathematical correction for isobaric interferences, selection of measurement mode for each analyte, and analytical sequence set up was simplified by the Qtegra ISDS Software. The LabBook file format, which provides all relevant information regarding the acquired data of the sequence with method information, data evaluation settings, and quality control details, helps users to easily access all information in one place.

Results and discussion

Linearity

Excellent linearity ($R^2 > 0.999$) was observed for all target elements across the calibration range. An example calibration curve for cadmium (Cd) is shown in Figure 1 (0.05 to 20 $\mu\text{g}\cdot\text{L}^{-1}$). Also, the repeatability of the raw intensity of three subsequent measurements for each standard solution was typically observed in the range of 0.4 to 3.3% (RSD).

The instrumental detection limits (IDL) for each analyte were automatically calculated in the Qtegra ISDS Software. All analytical figures of merit, such as linearity and IDL, are summarized in Table 4.

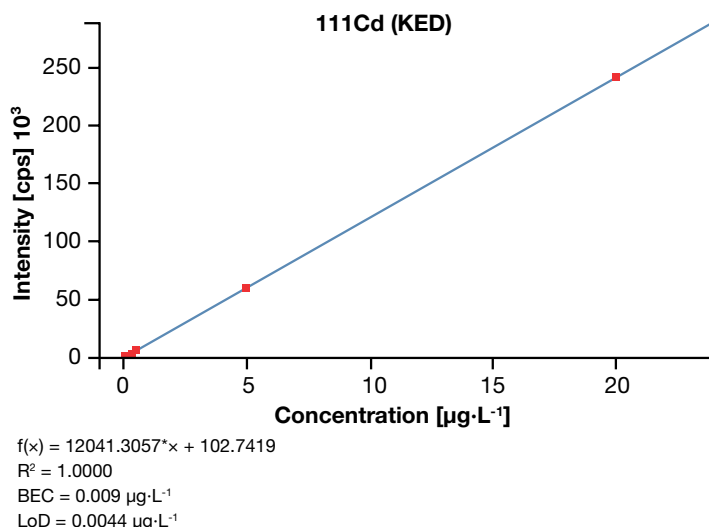


Figure 1. Calibration curve of cadmium (Cd) obtained from Qtegra ISDS Software over a concentration range of 0.05 to 20 $\mu\text{g}\cdot\text{L}^{-1}$

Table 4. List of elements, internal standards, isotopic mass, calibration range, R^2 , and instrument detection limit (IDL)

Element (symbol)	Mass	IS element	Mass	Calibration range ($\mu\text{g}\cdot\text{L}^{-1}$)	R^2	IDL ($\mu\text{g}\cdot\text{L}^{-1}$)
Lithium (Li)	7	Scandium (Sc)	45	2–200	0.9999	0.0137
Beryllium (Be)	9	Scandium (Sc)	45	2–200	0.9999	0.0183
Boron (B)	11	Scandium (Sc)	45	2–200	0.9999	0.2090
Vanadium (V)	51	Scandium (Sc)	45	2–200	0.9999	0.0149
Chromium (Cr)	53	Scandium (Sc)	45	2–200	0.9999	0.1620
Cobalt (Co)	59	Scandium (Sc)	45	0.05–20	0.9999	0.0008
Nickel (Ni)	60	Scandium (Sc)	45	2–200	0.9999	0.0081
Copper (Cu)	65	Scandium (Sc)	45	2–200	0.9999	0.0207
Zinc (Zn)	66	Scandium (Sc)	45	50–2000	0.9999	0.0001
Arsenic (As)	75	Germanium (Ge)	72	0.1–20	0.9999	0.0045
Selenium	77	Germanium (Ge)	72	0.1–20	0.9998	0.1350
Molybdenum (Mo)	98	Rhodium (Rh)	103	2–200	0.9987	0.0008
Cadmium (Cd)	111	Rhodium (Rh)	103	0.05–20	0.9999	0.0044
Tin (Sn)	118	Rhodium (Rh)	103	0.05–20	0.9999	0.0009
Antimony (Sb)	121	Indium (In)	115	0.1–20	0.9999	0.0014
Barium (Ba)	137	Terbium (Tb)	159	2–200	0.9998	0.0041
Mercury (Hg)	202	Bismuth (Bi)	209	0.05–20	0.9994	0.0030
Lead (Pb)	208	Bismuth (Bi)	209	0.1–20	0.9999	0.0072

Method performance

To check the incurred value of selected turmeric, black pepper, and red chilli powder samples, the microwave digested samples were analyzed using KED mode for interference removal for all elements. The obtained values are given in Table 5. Noticeably, for most of the elements, the concentrations in the samples were below the limit of quantification BLQ.

Table 5. Determined values in mg L⁻¹ for all analyte elements in turmeric powder (TP), black pepper powder (BPP), and red chilli powder (RCP) samples

Element	TP-1	TP-2	BPP	RCP-1	RCP-2	RCP-3
Li	BLQ	BLQ	BLQ	0.4	0.5	0.5
Be	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ
B	3.3	3.7	12.8	12.9	12.8	13.6
V	0.5	0.9	BLQ	0.4	0.5	BLQ
Cr	0.5	BLQ	1.3	0.5	0.4	BLQ
Co	0.2	0.3	BLQ	0.6	0.7	0.6
Ni	0.5	0.5	2.0	0.8	1.1	0.9
Cu	3.7	4.3	15.7	7.4	8.5	8.8
Zn	BLQ	13.0	BLQ	BLQ	BLQ	BLQ
As	BLQ	0.03	0.02	0.03	0.04	0.03
Se	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ
Mo	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ
Cd	0.03	0.03	0.02	0.07	0.08	0.06
Sn	0.03	0.01	BLQ	0.02	0.02	BLQ
Sb	BLQ	BLQ	0.01	BLQ	BLQ	BLQ
Ba	11.5	5.9	76.6	9.0	6.3	5.1
Hg	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ
Pb	0.18	0.09	0.1	0.16	0.17	0.12

Limit of quantification (LOQ)

The limit of quantification (LOQ) of all target elements was calculated by aspirating 23 method blanks prepared as described in the sample preparation section and analyzed for all target elements, as described in the AOAC 2015.01 guideline. The standard deviation was calculated for the response of all method blanks. The limit of quantification (LOQ) was calculated as six times the standard deviation of the response of each target element. The observed LOQs were verified in the turmeric powder sample by spiking at the desired concentration in decreasing order. Those spiked levels that offered greater than 80% recovery with <10% RSD were considered as LOQs. The observed LOQs are given in Table 6. The LOQ values include the total dilution factor, which was involved in sample preparation.

Accuracy and precision

To demonstrate the performance of the optimized protocol, a total number of 18 samples were spiked at three different levels, i.e., L1 (LOQ), L2, and L3 in turmeric powder. The percent recoveries and precision were calculated for each spiked concentration (n=6) and are given in Table 6. The observed recoveries were in the range of 80 to 115% with RSD <10%, which is well within the criteria set by AOAC2015.01.

Table 6. Spiked concentrations at three different levels for recovery (%) and precision (% RSD), Level 1 (L1) (LOQ), Level 2 (L2), and Level 3 (L3), in mg·kg⁻¹ in turmeric powder

Element	L1 (LOQ)			L2			L3		
	Spiked concentration (mg·kg ⁻¹)	% Rec.	% RSD	Spiked concentration (mg·kg ⁻¹)	% Rec.	% RSD	Spiked concentration (mg·kg ⁻¹)	% Rec.	% RSD
Li	0.4	87	3.4	0.8	88	2.7	20	85	1.7
Be	0.4	92	4.9	0.8	88	3.8	20	87	2.2
B	0.4	98	1.7	0.8	89	2.3	20	87	2.3
V	0.4	98	2.6	0.8	85	2.9	20	91	2.5
Cr	0.4	84	1.7	0.8	87	1.6	20	88	1.9
Co	0.125	99	8.0	0.25	105	4.0	1.0	100	6.0
Ni	0.4	97	3.0	0.8	94	1.4	20	93	1.3
Cu	1.5	95	8.0	3.75	92	6.0	30	92	3.0
Zn	10.0	98	3.0	20.0	95	1.3	200	95	1.2
As	0.02	99	5.3	0.04	95	2.5	2.0	96	1.6
Se	0.4	110	4.4	0.8	112	4.6	20	110	2.2
Mo	0.4	103	3.0	0.8	108	5.2	20	97	5.1
Cd	0.01	86	2.9	0.02	93	6.2	1.0	91	5.0
Sn	0.01	82	4.9	0.02	89	4.1	1.0	94	4.7
Sb	0.02	110	4.8	0.04	107	1.2	2.0	105	1.9
Ba	0.8	99	1.4	2.0	102	1.2	20	101	1.5
Hg	0.02	91	1.4	0.05	98	0.4	1.0	112	1.1
Pb	0.02	98	4.4	0.04	107	3.5	2.0	99	1.4

To increase the scope of the method, the optimized method was applied to black pepper and red chilli powder samples. Both sample types were assessed for the incurred elemental concentration. With that, a black pepper sample was spiked at one level (L3) in six replicates as quality

control to check the overall performance of the optimized analytical method. The average recoveries observed in the black pepper were in the range of 81 to 112% with <6.3% RSD for 19 elements (Figure 2).

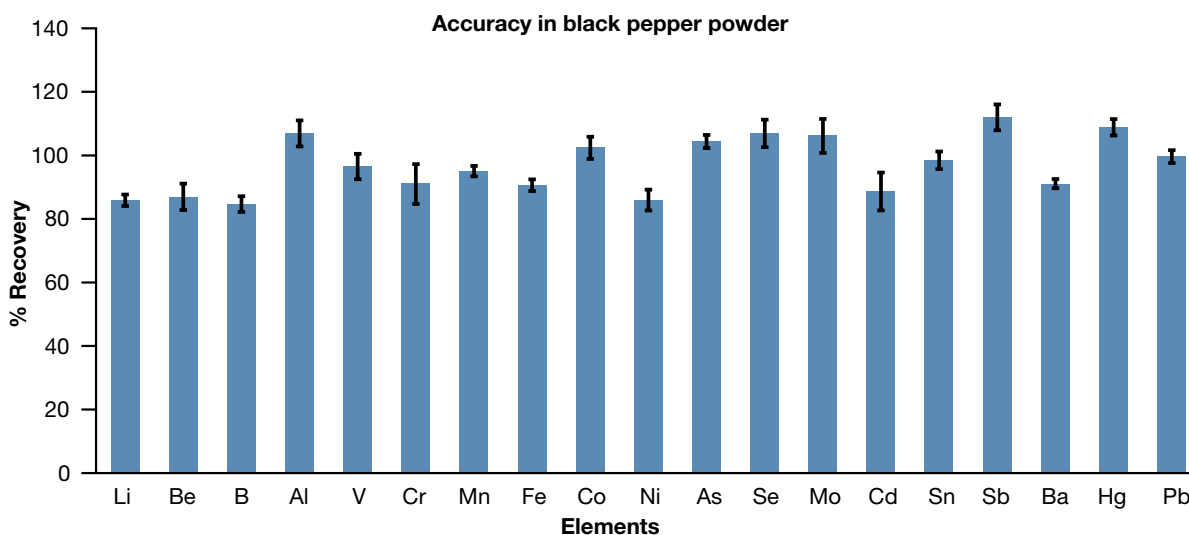


Figure 2. % Recovery and % RSD observed in spiked black pepper powder samples

Method robustness

Though the samples were appropriately digested, they are still prone to influences such as signal suppression or enhancement with the presence of matrix constituents, such as dissolved solids like sodium, calcium, or residual carbon. The behavior of Internal standards is considered as an indicator of suppressed or enhanced signal intensities in the presence of matrix effect. The internal standards were selected to best match analytes in terms of m/z and ionization potential to monitor and correct for potential signal drift, which could occur due to the matrix effect.

The % recoveries of internal standards were calculated using the Qtegra ISDS Software and values were compared with the criteria set by AOAC. 2015.01.

The response of internal standards during a sequencing run of spice samples ($n=45$) showed excellent robustness. The internal standard elements showed recoveries in the range of 80 to 110% against the criteria of 60 to 125% (AOAC 2015.01). with the precision of <10% RSD. Recoveries of Sc, Ge, In, Rh, Tb, and Bi, which were used as internal standards, are shown in Figure 3.

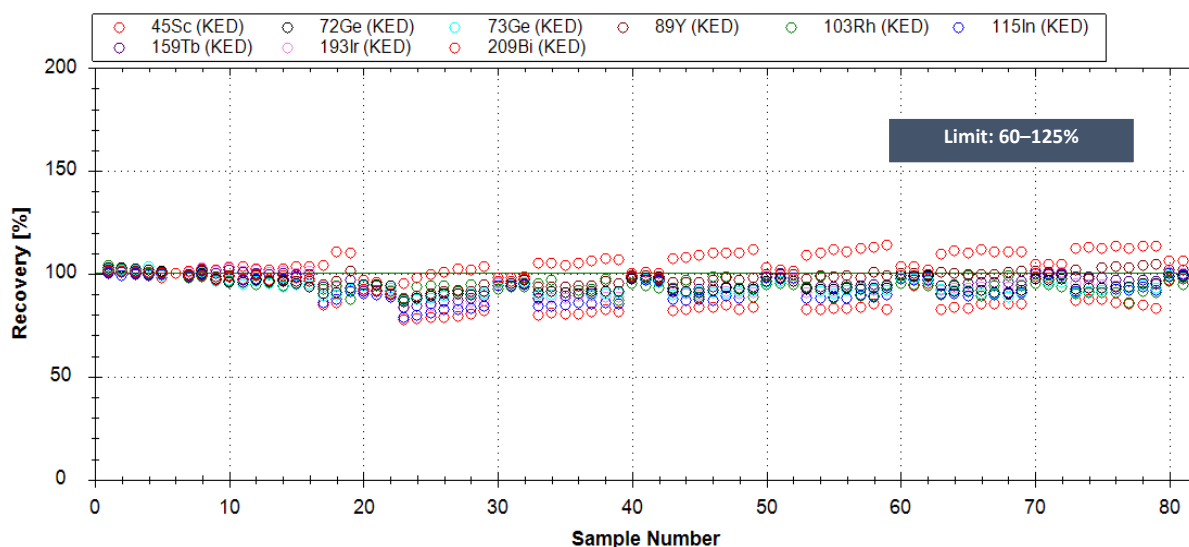


Figure 3. % Recovery of internal standards in an analytical sequence containing standards, matrix samples, and quality controls (>80 samples) acquired continuously over 4.5 hours

Continuing calibration verification standards (CCV) were examined for all elements after every six spiked samples to assess the accuracy of the analytical sequence. A standard (std 3) from linearity solutions was aspirated and recoveries were calculated against their initial concentrations using Qtegra ISDS Software. The obtained recoveries were well

within the acceptance criteria of 85 to 115% set as per AOAC 2015.01, which indicates that there is no significant drift in the intensities and calculated concentrations beyond the limit of 85 to 115% and it ensures the quality of analytical data. The observed percentage recoveries of all elements are shown in Figure 4.

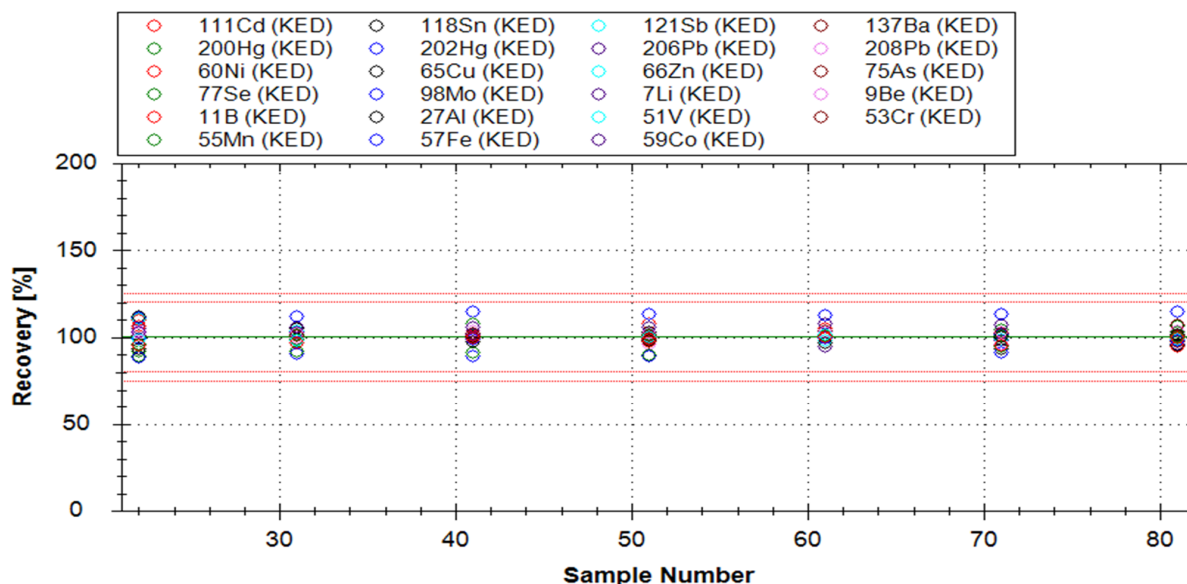


Figure 4. % Recovery of CCV standards across an analytical sequence with >80 samples. Red lines indicate the tolerance limits ($\pm 10\%$ and $\pm 15\%$).

Conclusion

- The experiments were performed to demonstrate the suitability of the iCAP-RQ ICP-MS system for the analysis of trace elements in spices using a simplified, high throughput sample preparation procedure.
- The analytical method described met all the performance criteria (accuracy, precision, and quality control) as specified in AOAC 2015.01.
- The data obtained for internal standards response and intermittently measured QC standards indicate that this analytical approach offers consistent performance over long analytical sequences.
- The proposed analytical workflow offers a complete, robust analytical solution for analytical laboratories with high sample throughput for trace level multielement analysis in spices using iCAP RQ ICP-MS.

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