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**Method Development for the Determination of Elements in *Hypericum perforatum* L.
(St John's wort) Herb and Preparations using ICP-OES and Microwave Digestion**

Jade D. Owen, Sara J. Evans, Amira Guirguis, Katherine Kellett, and Jacqueline L. Stair*

School of Life and Medical Sciences
Department of Pharmacy, Pharmacology and Postgraduate Medicine
University of Hertfordshire
Hatfield, UK

***Correspond to:**

Dr. Jacqueline L. Stair
Department of Pharmacy,
Pharmacology and Postgraduate Medicine
University of Hertfordshire
College Lane,
Hatfield, AL10 9AB, UK.
Email: j.stair@herts.ac.uk
Tel: +44 (0)1707 284000 Ext: 3367
Fax: +44 (0)1707 284870

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Abstract

A method was developed to analyse St John's wort (*Hypericum perforatum* L.) herb and preparations using Inductively Coupled Plasma – Optical Emission Spectroscopy (ICP-OES) to determine the quantity of 11 elements (Al, B, Ba, Ca, Cu, Fe, Mg, Mn, Ni, Sr, and Zn). This study includes the evaluation of digestion acids and calibration methods, as well as instrumental parameters such as choice of nebuliser and emission wavelength. Two nebulisers (Conikal and SeaSpray) performed similarly for most elements and two optimum wavelengths were determined for each element. Five acids were evaluated for the digestion of the Polish Certified Reference Material Tea Leaves (INCT-TL-1) while three were taken forward to use for the different St John's wort formulations (i.e., herb, capsule and tablet). A simple protocol using 5 ml HNO₃ was sufficient in most cases, however variability was observed for elements often bound in silicates (e.g., Al, Fe, and Zn). An external weighted calibration was also found to be preferential over unweighted, and the use of standard addition affected some concentration values up to 20%. Therefore, this paper presents the development and optimised method parameters to be used with ICP-OES that will allow the analysis of 11 key elements present in St John's wort herb and preparations.

Introduction

Herbal medicines are becoming increasingly popular as an alternative to synthetic mainstream medicines. However, due to their complex nature adverse effects can be seen including interactions with other medications, allergic reactions and poisoning from metal contamination [1, 2]. Therefore, the characterisation of both molecular and elemental species in herbal material is important for quality control measures. More recently, the elemental profiling of herbs has shown promise as an additional tool for quality assessments [3]. For this type of work, the analysis of elements in herbs can be particularly challenging, as it is difficult to obtain homogenous and representative samples with minimal contamination. Thus, sample preparation and analysis methodology is essential to ensure data produced is a true representation of the herbal medicine.

St John's wort (SJW) is a popular medicinal plant utilised for the treatment of mild to moderate depression [4]. It is also known to be a metal accumulator of elements such as cadmium [5] and elements present in the growing medium such as nickel [6], lanthanum [7] or chromium [8] can affect the production of bioactive compounds. A variety of preparation techniques have been reported for the elemental analysis of SJW herb and preparations. Although acid digestion is the most common technique, other sample preparation parameters can vary drastically between studies. Some studies use one [9-12], two [13-19] or three [10, 11, 20] acids (e.g., HNO₃, HCl, H₂O₂, HClO₄ or HF) for the digestion of samples where the volumes and ratios used can vary considerably. The acid digestion approach also differs with studies utilising hotplate [12, 17, 18, 21], sand bath [22], autoclave [16], and microwave digestion [9, 15, 19, 20]; the latter being a quicker method and generally producing more consistent results. The presence of silica in SJW samples may also affect the elements recovered [23]. The majority of pre-existing SJW studies [13, 15, 22, 24-29] often focus on 5 elements or less, while Bu et al [20] monitored 12 and our recent investigations found 15 elements in SJW preparations [3]. Due to the lack of Certified Reference Materials (CRMs) available for SJW, a number of plant based CRMs have been reported for accuracy assessments including tomato leaves [18, 20, 30], peach leaves [20], spinach leaves [20], tea leaves [3, 9, 19, 31], mixed polish herbs [31], grass [32], and tobacco [26].

In addition to sample preparation, the method of detection is also a key aspect for quantitative analysis of diverse elements in SJW to enable elemental profiling. Instruments such as Atomic Absorption Spectroscopy (AAS) and Graphite Furnace Atomic Absorption Spectroscopy (GFAAS) [9-11, 13-16, 18, 21, 22, 25, 26, 28, 29, 33-36] have been widely used, however the potential to form refractory compounds is higher and the calibration range is often limited to a dynamic range of order 10²-10³ [37]. A smaller number of studies employed Hanging Mercury Drop Electrode (HMDE) [32],

Thin Mercury Film Electrode (TMFE) [17], Direct Mercury Analyser (DMA) [30] or Laser Ablation – Inductively Coupled Plasma – Mass Spectrometry (LA-ICP-MS) [20]. Other studies have used Inductively Coupled Plasma – Optical Emission Spectroscopy (ICP-OES) [11, 17, 18, 27, 38, 39] and Inductively Coupled Plasma – Mass Spectrometry (ICP-MS) [9, 12, 19, 20, 24, 40] as ICP methods have been highlighted by the US pharmacopoeia [41] for element quantification in pharmaceutical-like substances due to the higher temperatures used (i.e., reduction of chemical interferences), low detection limits, a wide dynamic range (up to order 10^6) and simultaneous detection capabilities [37]. Although ICP-MS offers lower detection limits, ICP-OES can often be a more economical choice. The method of calibration is also important due to the trace levels often examined and complex matrix of SJW. In previous studies an external calibration method is usually indicated [10-15, 17-22, 27, 31, 35, 42]. One study used standard addition for the analysis of Pb [14] and a small number of studies used an internal standard [12, 19, 20], often with ICP-MS.

Due to the diversity of sample preparation and instrumental parameters reported in the literature thus far for the analysis of elements in SJW, it is necessary to evaluate systematically which methods will indeed be suitable for a large number of elements and diverse formulations for future applications (e.g., elemental profiling). Thus, this paper describes a systematic method development for 11 key elements (i.e., Al, B, Ba, Ca, Cu, Fe, Mg, Mn, Ni, Sr and Zn) present in SJW herb and preparations. Aspects of the sample preparation, ICP-OES parameters and calibration were investigated and optimised for the creation of elemental profiles.

Method

Materials

All labware was acid washed overnight with 4M nitric acid and rinsed thoroughly with deionised water before use. Nitric acid 70 % (99.999 % trace metal basis), hydrochloric acid 37 % (99.999 % trace metal basis), hydrogen peroxide 35 % (for trace analysis) and ammonium fluoride (99.99 % trace metal basis) (Sigma-Aldrich) were used for acid digestion. Elemental stock solutions, 1000 ppm of Al, B, Ba, Mg, Mn, and Ni (Fisher), Ca, Cu, Fe, Sr and Zn (Merck), were used to prepare calibration standards. Millipore 0.22 μm syringe filters were used to filter the samples before analysis by ICP-OES. Polish Certified Reference Material (CRM) Tea Leaves (INCT-TL-1) was used in development and validation. Samples of *Hypericum perforatum* L. (St John's wort, SJW) including raw herb, tablet and capsule, which used aerial plant parts such as flowers, leaves, and stems, were sourced from retail and internet suppliers (Table S1 in Supplementary Information).

ICP-OES Method Investigation for Multi-element Analysis of SJW

Elemental analysis was carried out using a Varian 710-ES ICP-OES with SPS3 autosampler. Optimisation of the ICP-OES parameters were conducted as per the manufacturer's recommendations [43]. A total of up to six wavelengths (Table 1) were compared for each element to examine their limit of detection (LOD) and identify any possible interference between elements in the multi-element standard. The two most suitable wavelengths were then selected. Following this, two nebuliser types were compared by evaluating the LODs; a Conikal nebuliser which is used for general elemental analyses and a SeaSpray nebuliser which is adapted to high salt content samples. A blank sample of 2 % HNO₃ was analysed 20 times on three separate days. The standard deviation of the 20 blank measurements was then calculated and the value was multiplied by 3 for LOD and by 10 for limit of quantification (LOQ). The LOD and LOQs were calculated for each day and a mean and standard deviation were calculated between the three days. Comparison of the nebulisers and resultant LODs was carried out by applying a comparison of means two-tailed t-test (95% confidence) in Excel. A calibration curve for each element was constructed using both an unweighted and weighted regression. The differences between the actual concentrations of the lowest three standards were compared to their calculated concentrations from either an unweighted or weighted regression line. The percentage error from the three lowest concentration standards was added cumulatively for comparison.

Sample Preparation Investigations

Comparison of Different Acids

Acid digestion was carried out using a Mars Xpress microwave (CEM Corporation) with Teflon digestion vessels. A total of five digestion acid mixtures (AM) were used for the study based on those recommended by CEM, seen commonly in literature [12, 14, 16, 31, 34, 42, 44-52], or recommended by the British Pharmacopeia [53] for the digestion of plant material. The acid mixtures were AM 1 (5 ml nitric acid), AM 2 (2 ml of water, 8 ml nitric acid and 2 ml hydrogen peroxide), AM 3 (2 ml of water, 8 ml nitric acid, 2 ml hydrogen peroxide and 200 mg ammonium fluoride), AM 4 (2 ml of water, 8 ml nitric and 2 ml hydrochloric acid) and AM 5 (15 ml nitric acid). All five acids were evaluated for the digestion of the trace element CRM used for the study, Polish CRM Tea Leaves (INCT-TL-1). Acid mixtures 1-4 were then evaluated for the digestion of a SJW herb, tablet, and capsule (see Supplementary Information Table S1 for sample information for H1, C1, and T1). For all analyses, approximately 0.4 g of sample was digested with acid in triplicate using the following program: heat over 12 min to 160 °C, hold for 2 min, heat to 175 °C over 2 min, hold for 2 min and finally heat to 185 °C over 2 min and hold for 15 min then allow to cool. The samples were then diluted 1:10 with deionised water, centrifuged at 9000 rpm for 45 min and syringe filtered (0.22 µm). The samples were

then analysed via ICP-OES. The effect of grinding in a pestle and mortar on transfer contamination was also investigated (presented in Supplementary Information). Comparison of AM 1 and 3 on SJW was carried out by applying a comparison of means two-tailed t-test (95% confidence) in Excel.

Matrix effects

Matrix effects of different preparations were evaluated using standard addition with a different SJW herb, tablet and capsule preparation (see Supplementary Information Table S1 for sample information for H2, C2, and T2. The samples were artificially enriched with 2.5 ppm for elements B, Ba, Ca, Cu, Mg, Ni, Sr, and Zn, 5 ppm Fe and Mn, 25 ppm for Al, 60 ppm Mg and 225 ppm Ca. Each element was added at concentrations at least five times the sample concentration [54]. The SJW test sample and one level of added analyte was used with repeated measurements (n=3) where the concentration was determined by extrapolating to the zero response using a linear regression.

Results and Discussion

ICP-OES Method Investigations for Multi-element Analysis of SJW

For each of the 11 elements, up to six emission wavelengths were inspected and two selected which had relatively low LODs and were free of spectral interferences (Table 1). Two wavelengths were monitored per element in case of spectral interferences arising from a particular sample, however less sensitive emission lines were used for Ca (370.602 and 422.73 nm) and Mg (278.142 nm) for concentrations higher than 20 and 10 ppm, respectively. Comparison of LODs for the two nebuliser types showed no significant difference between most elements (Table 2), however the SeaSpray had statistically significant ($p < 0.05$ t test) lower LODs for Mn and Zn. The SeaSpray nebuliser is also designed for high salt content samples, thus a good choice for analysis of herbal formulations and was used for the following experiments. Calibration curves for the 11 elements were constructed using both an unweighted and weighted regression. As many elements are present at trace level in SJW, the three lowest calibration points were evaluated for their accuracy. The results (Table 3) show that for the majority of elements, the weighted regression line had less error at low concentrations in comparison to an unweighted regression. For example, with a weighted regression Al, Ba, Mn, and Sr had cumulative uncertainties of 10, 11, 4 and 10 %, whereas with an unweighted regression these values were 36, 25, 18, and 46 %, respectively. Unweighted regression lines will often be dominated by the higher concentrations whereas in weighted regression the calibration line passes more closely by the lower concentration data points. Therefore a weighted calibration was deemed appropriate when analysing these 11 elements for SJW samples.

Sample Preparation Optimisation

Comparison of Different Acids

Due to the dearth of appropriate CRMs for SJW herbal material, the Polish CRM (INCT-TL-1), tea leaves, was chosen based on its physical similarity to SJW (i.e., leaves and stem), the elements selected for certification and correlation to prevalence in SJW samples, and its relative affordability [3, 9, 31]. The suitability of using the CRM was investigated by first determining the recovery for the certified elements using a range of acids. All digested tea leaves were visually clear indicating good digestion. The concentrations obtained with each acid were compared to the certified values (Figure S1 Supplementary Information) and showed very similar results across the five acid solutions where recovery values for most elements were above 80 %, a typical requirement for recovery studies [41]. The most notable difference was when using AM 3, where the mean B concentration was 3x higher than certified values with poor reproducibility; this is likely due to the HF produced causing leaching of B from glassware. The use of a HF producing digestion acid for the CRM was unnecessary as the other four acids performed similarly for the remaining elements, however this will be reviewed for SJW. Also, the use of 10 ml additional HNO₃, when comparing AM 1 and 5, did not seem necessary for the amount of substance evaluated (ca., 0.4 g) as no marked improvements in concentration resulted. For these reasons, AM 1, 2, and 4 were deemed suitable acids for the CRM digestion. Although the CRM was certified for 11 elements, we have previously reported on the use of spiked addition for adding additional elements of interest [3].

As mentioned above, it was of interest to initially investigate the effect of an HF producing acid on the digestion of silicates present in SJW as this may be different to the CRM. A SJW herb sample was digested using AM 1 and 3 and the elemental concentrations compared. Results showed that the majority of elements were consistent between the two acids with no significant difference ($p < 0.05$ t test) with the exception of Al, B and Fe, which increased 104, 28, and 27 %, respectively, using AM 3. Therefore, the presence of silicates with associated elements seems greater in the SJW herb than in the CRM selected for the study. Gomez et al. [10, 11] reported using five drops of HF in order to digest silica salts in SJW samples before analysis. Although concentration increases were seen for Al, B and Fe, the use of HF is often avoided due to the potential risk to glass components and health. Therefore, depending on the nature of the study and if the silicate composition is desired, the use of other acids may be sufficient for further analysis.

In order to investigate the effect of AM 1, 2, and 4 on the digestion of SJW, the element concentrations of a herb, capsule and tablet were compared (Table 4). Initially, when comparing the elemental

composition of the SJW formulations, there are notable differences between the formulations reflecting the composition. For example, the tablet formulation contains ground herb while the capsule contains SJW extract. Thus, many of the element concentrations are similar for the herb and tablet, while some of the elements (i.e., Al, Ba, Ca, and Fe) are an order(s) of magnitude lower for the capsule. Although the use of an herbal extract can decrease the element concentration from the herb source, the addition of excipients is an external source of elements. This can be seen for the Mg concentration in the capsule which is comparable to that of the herb and tablet as magnesium stearate was an added excipient for the particular capsule evaluated. A more comprehensive discussion of these differences between formulations is presented in a recent study.[3] Due to these differences in the plant material and added excipients, it was of interest to investigate the digestion of these different formulations with a range of acid mixtures. When comparing the efficacy of the different acid solutions for digestion of a herb, capsule and tablet, the concentrations were within 20 % or less when comparing across the AMs for each formulation. Greater than 20 % concentration variation across the AMs was consistently observed for Al, Fe and Zn, which again may be attributed to the silicates present and the degree of digestion [10, 11]. Interestingly, these results indicated that the use of AM 1 increased the recovery of Al, Fe, and Zn, up to 50 %, and on occasion for Ca (capsule) and Cu (herb). This may be due to the final acid digestion concentration of HNO₃ as the HNO₃ concentration is diluted when adding additional modifiers for AM 2 and 4. Therefore, the results suggest that conc. HNO₃ with no additional modifier is a suitable acid for digestion of SJW herb, capsules and tablet formulations.

i. Matrix effects

Due to the range of SJW preparations available, the effect of the matrix on the concentration of elements was evaluated using standard addition on second set of comparable SJW samples. The elemental concentrations determined using standard additional were compared to those determined by external weighted calibration. The results (Table 5) show that for the SJW herb, capsule and tablet, the weighed calibration results are, on average, within 13, 20 and 22 %, respectively, of the standard addition results. The US pharmacopoeia states recoveries of ± 20 % are acceptable for elements in pharmaceuticals [41]. Overall the standard addition results are higher than those observed for the external calibration, therefore this indicates that SJW samples do display matrix effects and in particular the processed preparations. However, the use of standard addition can be time consuming and requires additional sample, thus this would need to be carefully considered when choosing a calibration method for the quantification of elements in SJW preparations.

Conclusions

In this paper a number of method parameters were evaluated for the accurate determination of 11 elements in SJW herb and preparations using ICP-OES. For a multi-element determination of SJW formulations, a SeaSpray nebuliser is recommended due its ability to deal with high salt content without compromising LODs. For Ca and Mg, less sensitive emission lines are often necessary as these elements can be present in SJW samples in elevated concentrations. Also the use of a weighted calibration was found to be suitable for the majority of elements investigated at trace level. Although a number of acids were investigated, the use of HNO₃ was suitable in most cases for the digestion of both the CRM and SJW preparations, indicating the selected CRM is an appropriate choice for multi-element detection in SJW preparations. However, there is an indication that HF will be needed if full digestion of silicates in SJW is desired, which may impact levels of Al, Fe, and Zn. The use of standard addition also suggested the matrix may affect concentration levels in SJW preparations up to 20 %. Thus, a number of method parameters have been recommended for evaluation of elemental profiles for SJW herb and preparations.

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Tables

Table 1. Wavelengths examined for each element for limits of detection (LOD) comparison.

Element	Wavelength (nm)					
Al	237.312	257.509	394.401 ¹	236.705	308.215	396.152 ¹
B	182.577	208.889	208.956	249.678 ¹	249.772 ¹	- ²
Ba	233.527	455.403 ¹	493.408 ¹	585.367	614.171	- ²
Ca	315.887	317.933	370.602 ¹	393.366	396.847 ¹	422.673
Cu	213.598	224.700	324.754 ¹	222.778	223.009	327.396 ¹
Fe	234.350	238.204 ¹	239.563	259.940 ¹	260.709	261.187
Mg	279.078 ¹	279.553 ¹	279.800	280.270	285.213	383.829
Mn	257.610 ¹	259.372 ¹	279.482	260.568	293.931	- ²
Ni	221.648 ¹	222.295	232.138	216.555	230.299	231.604 ¹
Sr	215.283	421.552 ¹	460.733	216.597	338.071	407.771 ¹
Zn	206.200	334.502	472.215	202.548	213.857 ¹	330.258

¹wavelengths selected for analytical use.

²instrument only had 5 wavelength options for these elements.

Table 2. Limits of detection (LOD) comparison for SeaSpray and Conikal nebulisers using selected wavelengths.

Element	Wavelength (nm)	Limit of Detection (ppb) ^{1,2}			
		SeaSpray		Conikal	
		AVE	±1SD	AVE	±1SD
Al	396.152	5.1	0.5	5.1	0.4
B	249.772	7	2	9	1
Ba	455.403	0.21	0.03	0.17	0.01
Ca	396.847	1.9	0.1	1.2	0.6
Cu	327.395	4.5	0.7	5	1
Fe	238.204	1.2	0.1	1.6	0.3
Mg	279.553	0.24	0.05	0.15	0.04
Mn ³	257.61	0.23	0.01	0.28	0.01
Ni	231.604	3.4	0.6	4	1
Sr	407.771	0.06	0.01	0.06	0.01
Zn ³	213.857	0.63	0.03	1.1	0.1

¹ LOD = SD X 3; SD calculated from 20 replicate measurements on a single day.

² LODs reported are AVE ± 1SD calculated from three separate days (n=3).

³significant difference (p<0.05 t test) between nebulisers.

Table 3. Comparison of calibration error between weighted and non-weighted regression lines

Element	Wavelength (nm)	Total Cumulative Error % ¹	
		Weighted	Non-weighted
Al	396.152	10	36
B	249.772	28	13
Ba	455.403	11	25
Ca	370.602	14	26
Cu	327.395	2	3
Fe	238.204	26	23
Mg	278.142	34	37
Mn	257.610	4	18
Ni	231.604	3	4
Sr	407.771	10	46
Zn	213.857	87	42

¹ Cumulative (out of 300%) for the three lowest concentration standards above the LOQ

Table 4. Elemental Concentrations in SJW herb, capsule and tablet after microwave digestion using acid mixture 1, 2, and 4.¹

Element	Herb($\mu\text{g/g} \pm 1\text{SD}$)			Capsule($\mu\text{g/g} \pm 1\text{SD}$)			Tablet($\mu\text{g/g} \pm 1\text{SD}$)		
	AM1	AM2	AM3	AM1	AM2	AM3	AM1	AM2	AM3
Al	72 \pm 9	103 \pm 7	89 \pm 8	4.9 \pm 0.4	5.6 \pm 0.8	4.7 \pm 0.7	61 \pm 6	84.4 \pm 0.4	90 \pm 6
B	23.8 \pm 0.2	23 \pm 2	21.6 \pm 0.5	15.6 \pm 0.3	12.2 \pm 0.6	14 \pm 1	37 \pm 2	38.3 \pm 0.1	40 \pm 2
Ba	15.8 \pm 0.1	14.0 \pm 0.3	14.7 \pm 0.3	0.40 \pm 0.04	0.26 \pm 0.04	0.18 \pm 0.03	5.7 \pm 0.3	5.9 \pm 0.4	5.7 \pm 0.1
Ca	4160 \pm 30	3740 \pm 30	3950 \pm 50	1012 \pm 9	500 \pm 30	404 \pm 4	5660 \pm 40	5260 \pm 60	5500 \pm 200
Cu	11.6 \pm 0.2	9.6 \pm 0.7	4.3 \pm 0.2	12.6 \pm 0.7	11.9 \pm 0.2	11.6 \pm 0.2	9.3 \pm 0.3	8.0 \pm 0.1	8.0 \pm 0.5
Fe	110 \pm 20	56 \pm 2	61 \pm 6	19 \pm 1	9.2 \pm 0.5	8.2 \pm 0.3	500 \pm 30	242 \pm 9	270 \pm 30
Mg	1467 \pm 9	1230 \pm 20	1270 \pm 10	1780 \pm 20	1830 \pm 30	1760 \pm 20	1660 \pm 30	1790 \pm 30	1740 \pm 70
Mn	82.8 \pm 0.3	78 \pm 1	79.8 \pm 0.8	16.3 \pm 0.4	16.0 \pm 0.4	15.7 \pm 0.3	84.5 \pm 0.6	84 \pm 1	84 \pm 3
Ni	3.03 \pm 0.08	2.70 \pm 0.07	3.2 \pm 0.4	2.30 \pm 0.08	1.43 \pm 0.07	1.28 \pm 0.05	3.2 \pm 0.6	3.7 \pm 0.1	3.7 \pm 0.2
Sr	17.74 \pm 0.07	15.9 \pm 0.3	16.5 \pm 0.1	1.16 \pm 0.04	0.78 \pm 0.04	0.69 \pm 0.05	9.3 \pm 0.1	8.5 \pm 0.1	9.0 \pm 0.2
Zn	33.4 \pm 0.5	26 \pm 4	47 \pm 1	46 \pm 3	26 \pm 1	26 \pm 1	36 \pm 1	24.4 \pm 0.2	21.8 \pm 0.6

¹AM refers to acid mixture

Table 5. Comparison of SJW element concentrations using an external calibration and standard addition.

Element	Standard addition ($\mu\text{g/g} \pm 1\text{SD}$) ¹			Weighted calibration ($\mu\text{g/g} \pm 1\text{SD}$) ¹		
	Herb	Capsule	Tablet	Herb	Capsule	Tablet
Al	188 \pm 2	61 \pm 6	28.5 \pm 0.4	170 \pm 30	51 \pm 1	24.4 \pm 0.4
B	35.4 \pm 0.3	22 \pm 2	23.1 \pm 0.4	29 \pm 1	17 \pm 1	15.6 \pm 0.5
Ba	11.0 \pm 0.1	0.62 \pm 0.06	0.91 \pm 0.01	9.6 \pm 0.4	0.44 \pm 0.05	0.78 \pm 0.09
Ca	6190 \pm 60	94000 \pm 8000	- ²	6000 \pm 100	87000 \pm 2000	- ²
Cu	7.65 \pm 0.07	16 \pm 1	9.66 \pm 0.06	6.9 \pm 0.4	14.6 \pm 0.6	10.4 \pm 0.2
Fe	168 \pm 2	91 \pm 8	- ²	160 \pm 30	78 \pm 1	- ²
Mg	1730 \pm 20	93 \pm 8	- ²	1510 \pm 70	60 \pm 10	- ²
Mn	124 \pm 1	12 \pm 1	16.0 \pm 0.2	115 \pm 3	10.9 \pm 0.2	15.4 \pm 0.2
Ni	1.66 \pm 0.02	2.2 \pm 0.2	1.84 \pm 0.03	1.30 \pm 0.04	1.65 \pm 0.09	1.50 \pm 0.02
Sr	21.7 \pm 0.2	28 \pm 2	6.5 \pm 0.1	17.80 \pm 0.04	21.8 \pm 0.5	5.64 \pm 0.04
Zn	31.1 \pm 0.3	44 \pm 4	31.5 \pm 0.5	25.0 \pm 0.6	35 \pm 1	25.1 \pm 0.6

¹ $\pm 1\text{SD}$ = ± 1 standard deviation

² outside calibration range

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