Analytical Methods

Analysis of non-fat soybean powder for the mass fractions of three elements: Copper and zinc by isotope dilution ICP–MS and calcium by ICP–AES

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ARTICLE INFO

ABSTRACT

Isotope dilution inductively coupled plasma–mass spectrometry with "approximate match" approach was developed for the measurement of mass fractions of Cu and Zn. An "approximate match" (within 10%) was made between calibration and sample blends. The method was validated against two certified reference materials, namely NIST SRM 1515 (Apple Leaves) and IAEA-359 (Cabbage). Inductively coupled plasma-atomic emission spectrometry with bracketing technique was developed for the measurement of mass fraction of Ca. NIST RM 8412 (Corn Stalk) was employed for method validation purposes. The validated methods were applied to the measurement of Cu, Zn and Ca in a sample of non-fat soybean pertaining to the international comparison CCQM-P64. The results submitted for the comparison were in excellent agreement with the mean values of all participants' results. Relative standard uncertainties of about 4% (k = 2, which gives a level of confidence of approximately 95%) were achieved in the measurement of the three elements concerned.

1. Introduction

Soybeans are leguminous plants native to eastern Asia. They are grown for their seeds that are rich in nutritional ingredients, like protein (~40%), oil (~20%), carbohydrate (~35%) and essential elements. Their main uses are for the production of oil, meal, flour, infant formula, etc. Soybean oil is the largest source of vegetable oil. To produce soybean oil, soybeans are cracked, rolled into flakes and extracted by solvents. Soybean meal is the material left after solvent extraction of flakes and has been widely used as livestock feed. As shown in the latest "Soybean Success 2005–2006 Report" (Available from: http://www.soygrowers.com/publications/ASA0506report.pdf) prepared by the American Soybean Association (ASA), some interesting figures relating to US soybeans are noteworthy. A bushel of soybeans, which weighs 60 pounds, can yield about 11 pounds of oil, 48 pounds of meal, or 1.5 gallons of biodiesel. The ASA reported that China purchased nearly 435 million bushels of US soybeans worth over $2.5 billion in 2005. Nowadays, the US is the world's largest producer and exporter of soybeans, whereas China is the world's biggest soybean importer. Not surprisingly, trading of soybeans is a multi-billion-dollar business. With a view to safeguarding good-quality products and ensuring fair trade practices, internationally accepted methods for assessing the nutritional ingredients in soybeans are definitely necessary. Soybeans are well known for their richness in essential elements, including copper, zinc and calcium. Cu is a component of enzymes for iron metabolism. The recommended dietary allowance (RDA) suggested by the Institute of Medicine of the National Academies for normal adults is about 0.9 mg Cu day^{-1} (Available from: http://www.iom.edu/Object.File/Master/7/294/Webtableminerals.pdf). Gastrointestinal distress and liver damage are problems associated with excessive intake of Cu. Zn is a component of multiple enzymes and proteins. It involves in the regulation of gene expression. The RDA for adults is 8–11 mg Zn day^{-1}. Higher amounts of Zn are needed during pregnancy and lactation. Ca has several important functions in human body, including bone and teeth formation, muscle contraction, blood clotting, and nerve pulse transmission. Deficiency of Ca leads to osteoporosis. However, excessive consumption will cause adverse effects such as kidney stones. The adequate intake for healthy adults is 1000–1300 mg Ca day^{-1}. Accurate determination of these elements can therefore provide an effective means of assuring the nutritional quality of soybeans.

The Government Laboratory of Hong Kong has been striving for the development of elemental analysis protocols for two main purposes: (i) certification of trace elements in food/plant samples as reference materials; and (ii) participation in international comparison exercises to enable global comparability of analytical results. To

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serve these purposes, we have successfully developed several protocols for the measurement of cadmium in oyster tissues (Yip, Chu, Chan, Chan, Cheung, & Sham, 2006) and iron in plant materials (Chu, Yip, Chan, & Sham, 2006) using isotope dilution mass spectrometry (IDMS). It is interesting to note that IDMS has been recognised by the Consultative Committee for Amount of Substance – Metrology in Chemistry (Comité Consultatif pour la Quantité de Matière, CCQM) to have the potential to be a primary method of measurement. The aim of the study was to develop protocols that were suitable for the certification measurement of Cu, Zn and Ca in plant samples. The instruments used were a quadrupole inductively coupled plasma–mass spectrometer and an inductively coupled plasma–atomic emission spectrometer. Both instruments are commercially available at affordable prices and are the norms in many analytical laboratories. Isotope dilution inductively coupled plasma–mass spectrometry (ID–ICP–MS) was employed for the measurement of Cu and Zn. The method was validated by the use of two certified reference materials (CRM), namely NIST SRM 1515 (Apple Leaves) and IAEA-359 (Cabbage). We did not apply IDMS in the measurement of Ca for two reasons. First, $^{40}Ca^+$, the most abundant isotope (abundance: 96.9%), suffers seriously from the isobaric interference due to $^{40}Ar^+$ when a quadrupole instrument is used for measurement. Second, instrumental sensitivities of minor Ca isotopes (i.e. $^{42}Ca^+$, $^{43}Ca^+$, $^{44}Ca^+$, $^{46}Ca^+$ and $^{48}Ca^+$) may not be sufficient for accurate isotope ratio measurement because of their low isotopic abundances. As such, inductively coupled plasma–atomic emission spectrometry (ICP–AES) was selected in the validation measurement of Ca in a reference material of NIST RM 8412 (Corn Stalk). The validated methods were finally applied to the measurement of Cu, Zn and Ca in a sample of non-fat soybean (abbreviated as CCQM soybean) pertaining to the CCQM-P64 pilot study.

2. Experimental

The experimental details are provided as Supplementary material (Aregbe & Taylor, 2003; Vocke, 1997).

3. Results and discussion

3.1. Determination of Cu and Zn using ID–ICP–MS

3.1.1. Possibility of spectral interferences in the CCQM soybean sample

Cu and Zn were quantified by measuring the isotope ratios of $^{63}Cu/^{65}Cu$ and $^{66}Zn/^{67}Zn$, respectively. The four isotopes concerned and various possible interfering species are shown in Table 1 (May & Wiedmeyer, 1998).

The amounts of Na, Ca and Mg vary from parts-per-million up to percentage levels in plant materials, like NIST SRM 1515 (Na: 24.4 mg kg^{-1}, Ca: 1.526%, Mg: 0.271%) and IAEA-359 (Na: 580 mg kg^{-1}, Ca: 1.85%, Mg: 0.216%). The polyatomic species $^{40}Ar^{23}Na^+$, $^{48}Ca^{16}O^1H^+$ and $^{40}Ar^{26}Mg^+$ interfere with the isotopes $^{63}Cu^+$, $^{65}Cu^+$ and $^{66}Zn^+$, respectively. It can be presumed that the influence of $^{48}Ca^{16}O^1H^+$ on $^{65}Cu^+$ is not significant because the abundance of ^{48}Ca (0.187%) is low. To check whether $^{40}Ar^{23}Na^+$

and $^{40}Ar^{26}Mg^+$ give significant interferences on the isotopes $^{63}Cu^+$ and $^{66}Zn^+$, respectively, the best way is to compare the measured isotope ratios in sample solutions with those in standard solutions for quadrupole based ICP–MS measurements.

Titanium stimulates the production of carbohydrates and encourages growth in plants. Most plants contain about 1 mg Ti kg^{-1}. As the abundances of ^{47}Ti (7.44%) and ^{49}Ti (5.41%) are quite low, the formation of interfering species of $^{47}Ti^{16}O^+$, $^{49}Ti^{16}O^+$ and $^{49}Ti^{16}O^1H^+$ can be expected to have less significant effect on the measurement of isotopes $^{63}Cu^+$, $^{65}Cu^+$ and $^{66}Zn^+$, respectively. As the abundance of ^{48}Ti (73.72%) is relatively high, attention should be paid to the formation of $^{48}Ti^{16}O^1H^+$ that interferes with the isotope $^{65}Cu^+$.

The formation of $^{130}Ba^{2+}$, $^{132}Ba^{2+}$ and $^{134}Ba^{2+}$ may affect the measurement of isotopes $^{65}Cu^+$, $^{66}Zn^+$ and $^{67}Zn^+$, respectively. Because of low abundances of ^{130}Ba (0.106%), ^{132}Ba (0.101%) and ^{134}Ba (2.417%) and of low formation of doubly charged ions (the instrument specification: $Ba^{2+}/Ba^+ < 3\%$), the effect of these interfering ions is considered negligible (estimated signal intensities for interfering ions: $^{130}Ba^{2+}$ (<200 cps), $^{132}Ba^{2+}$ (<100 cps), $^{134}Ba^{2+}$ (<100 cps); estimated signal intensities for analyte ions: $^{65}Cu^+$ (90,000 cps), $^{66}Zn^+$ (150,000 cps), $^{67}Zn^+$ (23,000 cps)).

The interferences induced by $^{31}P^{17}O_2^+$, $^{33}S^{16}O_2^+$ and $^{34}S^{16}O_2^+$ on the measurement of isotopes $^{65}Cu^+$ and $^{66}Zn^+$ should not be significant because the abundances of ^{17}O (0.038%), ^{33}S (0.76%) and ^{34}S (4.29%) are low. However, other interfering species $^{31}P^{16}O_2^+$ and $^{32}S^{16}O_2^1H^+$, which interfere with the isotopes $^{63}Cu^+$ and $^{65}Cu^+$, respectively, should be carefully evaluated.

Finally, Ar- and Cl-based interferences such as $^{40}Ar^{12}C_2^1H^+$, $^{35}Cl^{14}N^{16}O^+$ and $^{35}Cl^{16}O_2^+$ should be taken into consideration when the measurement of isotopes $^{65}Cu^+$ and $^{67}Zn^+$ is carried out.

High resolution ICP–MS is a prerequisite for the elucidation of the identities of interfering species at the four analytical masses (i.e. m/z 63, 65, 66 and 67, respectively). As identification of all possible interfering species was not of our prime concern, no further investigation was conducted. Nonetheless, the possibility of spectral interferences on the isotope ratios in the CCQM soybean sample was evaluated. The sequence of analysis was as follows: standard solution → sample solution of CCQM soybean. Each isotope ratio was measured twice. The measured isotope ratios (mean ± 1/2 absolute difference between duplicate values) in the standard solutions and the sample solution of CCQM soybean were found to be indistinguishable using a quadrupole ICP–MS (Table 2). The results suggested that that no additionally matrix based interfering species were formed at the four analytical masses concerned. Therefore, matrix separation using ion-exchange chromatography was not necessary. Similar findings were also observed in NIST SRM 1515 and IAEA-359.

3.1.2. Optimum blend isotope ratios

The error propagation factor, EPF$_{opt}$, is a theoretical optimum for spiking samples to achieve the best precision for isotope ratio measurement (Sargent, Harrington, & Harte, 2002). It is calculated from the isotope system being measured according to Eq. (1).

Table 1
Possible interfering species

Isotope	Na-based	Ca-based	Mg-based	Ti-based	Ba-based	P-based	S-based	Others
$^{63}Cu^+$	$^{40}Ar^{23}Na^+$			$^{47}Ti^{16}O^+$		$^{31}P^{16}O_2^+$		
$^{65}Cu^+$		$^{48}Ca^{16}O^1H^+$		$^{49}Ti^{16}O^+$	$^{130}Ba^{2+}$	$^{31}P^{17}O_2^+$	$^{33}S^{16}O_2^+$	$^{40}Ar^{12}C_2^1H^+$
				$^{48}Ti^{16}O^1H^+$			$^{32}S^{16}O_2^1H^+$	$^{35}Cl^{14}N^{16}O^+$
$^{66}Zn^+$			$^{40}Ar^{26}Mg^+$	$^{49}Ti^{16}O^1H^+$	$^{132}Ba^{2+}$		$^{34}S^{16}O_2^+$	
$^{67}Zn^+$					$^{134}Ba^{2+}$			$^{35}Cl^{16}O_2^+$

$$\mathrm{EPF_{opt}} = \sqrt{\frac{\text{Spike isotope abundance(enriched spike)}}{\text{Analyte isotope abundance(enriched spike)}} \times \frac{\text{Spike isotope abundance(sample)}}{\text{Analyte isotope abundance(sample)}}} \qquad (1)$$

Ideally, the blend isotope ratio should be equal to 1:1. Very low measurement uncertainties can be obtained because systematic errors in the determination of isotope ratios are cancelled out (García Alonso, 1995). However, when the error propagation factor is plotted as a function of the blend isotope ratio, a minimum is usually shown at a value that is not equal to one. In practice, the optimum blend isotope ratio is a compromise between the $\mathrm{EPF_{opt}}$ and the ideal 1:1 blend isotope ratio. Furthermore, it should be set in the range of 4:1–1:4 to maintain reasonable signal intensities for both analyte and spike isotopes (Watters, Eberhardt, Beary, & Fassett, 1997). In the present work, the $\mathrm{EPF_{opt}}$ values for the isotope systems of Cu and Zn used were found to be 12.3 and 1.8, respectively (Table 3). In order to strike a balance between systematic errors and signal intensities, the blend isotope ratios of $^{63}Cu/^{65}Cu$ and $^{66}Zn/^{67}Zn$ were therefore set in the range of 0.9–1 (Table 4).

3.1.3. Approximate match approach

Henrion (1994) proposed an approach in which a sample blend was exactly matched with an equivalent amount of a calibration blend. The advantages of the "exact match" approach negate many sources of errors such as mass bias and detector dead time. However, the procedure is time consuming and involves iterative preparations of calibration blends until a match is achieved. To simplify our procedure and retain the benefits of matching principle, "approximate match" approach (within 10%) was proposed in our work. The analysis of Cu in NIST SRM 1515 was illustrated as an example (Table 4). An "approximate "match" (within 10%) was made between the sample blend and the calibration blend with respect to (i) mass fraction of Cu (certified value $c_x = 5.64\ \mu g\ Cu\ g^{-1}$, $c_z = 5.6439\ \mu g\ Cu\ g^{-1}$); (ii) mass of the sample/primary assay standard solution ($m_x = 0.5004\ g$, $m_z = 0.5013\ g$); and (iii) mass of the spike ($m_y = 1.0267\ g$, $m'_y = 0.9993\ g$). As a result, K'_b (1.0711) and R'_b (0.9403) in the calibration blend matched well (within 10%) with K_b (1.0729) and R_b (0.9253) in the sample blend, respectively.

Table 2
Results of the measurement of $^{63}Cu/^{65}Cu$ and $^{66}Zn/^{67}Zn$ ratios (mean ± 1/2 absolute difference between duplicate values) in the standard solutions and the sample solution of CCQM soybean

Solution	
	$^{63}Cu/^{65}Cu$ ratio
Cu (20 µg L^{-1})	2.1353 ± 0.0020
CCQM soybean	2.1356 ± 0.0048
	$^{66}Zn/^{67}Zn$ ratio
Zn (100 µg L^{-1})	6.6550 ± 0.0471
CCQM soybean	6.6556 ± 0.0470

Table 3
$\mathrm{EPF_{opt}}$ values calculated for the isotope systems of Cu and Zn

Isotope	Abundance (%)	
	IUPAC	Spike solution
^{63}Cu (analyte isotope)	69.17	0.2926
^{65}Cu (spike isotope)	30.83	99.7074
$\mathrm{EPF_{opt}}$	12.3	
^{66}Zn (analyte isotope)	27.90	3.916
^{67}Zn (spike isotope)	4.10	89.510
$\mathrm{EPF_{opt}}$	1.8	

Following this approach, K'_b and R'_b in other calibration blends given Table 4 were found to match well with K_b and R_b in the corresponding sample blends. From our practical experience, the "approximate match" approach was sufficient to achieve the target relative expanded uncertainty of less than 5% ($k = 2$, which gives a level of confidence of approximately 95%) when using a quadrupole ICP–MS for measurement (see discussions in Section 3.4.).

3.2. Determination of Ca using ICP–AES

3.2.1. Optimisation of ICP–AES operating conditions

In order to maximise the efficiency of internal standardisation, the plasma parameters of ICP–AES should be optimised to obtain sufficient plasma robustness. The practice of monitoring the emission intensity ratio of Mg 280.271/Mg 285.213 was adopted (Mermet, 1998). Optimised plasma parameters, including RF power (1300 W) and flow rates of plasma, auxiliary and nebuliser (15, 0.5 and 0.8 L Ar min^{-1}, respectively), were set up. Ten repeated measurements of a standard solution containing 100 µg Mg L^{-1} were carried out. The value of Mg 280.271/Mg 285.213 was found to be (mean ± standard deviation) 5.7266 ± 0.0264. Precision (relative standard deviation, RSD) of less than 0.5% was obtained. Under the optimised conditions, the emission intensity ratio of Ca 317.933/Ca 315.887 was measured according to the bracketing sequence: standard solution A → sample solution → standard solution B. Precision (RSD) of less than 0.15% was obtained in the four sets of Ca 317.933/Ca 315.887 ratios (Table 5). It was concluded that all solutions were analyzed under robust plasma conditions, which fulfilled the acceptance criterion of RSD < 1%.

3.2.2. Internal standardisation and bracketing technique

Internal standardisation is suitable for overcoming instrumental drifts, flicker noises and signal variations due to matrix effects. In our study, the ICP WinLab software took the triplicate measurements of the emission intensities of the analyte and internal standard simultaneously and carried out the normalisation of the emission intensity of analyte with respect to that of internal standard in the calibration blank according to equations (S4)–(S6). There were three steps taken for calculation. First, the emission intensity of internal standard in the calibration blank "Intensity (IS, calib. blank)" was calculated as a mean value using equation (S4). Second, the emission intensity of analyte in the calibration blank "Intensity (analyte, calib. blank)" was corrected with a normalisation factor of "$\frac{\text{Intensity(IS, calib. blank)}}{\text{Intensity(IS, calib. blank},i)}$" using equation (S5). Third, the emission intensity of analyte in the analytical sample "Intensity (analyte, analytical sample)" was corrected with another normalisation factor of "$\frac{\text{Intensity(IS, calib. blank)}}{\text{Intensity(IS, analytical sample},i)}$" before subtracting the emission intensity of analyte in the calibration blank in accordance with equation (S6).

The response factors of standard solutions A and B, which were prepared to have concentrations covering the sample solution closely, were calculated using equations (S7) and (S8), respectively. The difference between the concentrations of standard solutions A and B should be ⩽200 µg Ca L^{-1}, which was the requirement set for bracketing technique. The concentration of the sample solution was obtained from the division of the method blank-corrected emission intensity of analyte in the sample solution by the average response factor according to equations (S9)–(S10). Finally, the

Table 4
Establishment of "approximate match" between sample and calibration blends

Symbol	Mass fraction of Cu (mg kg^{-1})			Mass fraction of Zn (mg kg^{-1})	
	CCQM soybean	NIST SRM 1515	IAEA-359	CCQM soybean	IAEA-359
c_z (µmol g^{-1})	0.2268	0.0888	0.0888	0.6880	0.5913
c_z (µg g^{-1})	14.4138	5.6439	5.6445	44.9899	38.6642
m_y (g)	1.0521	1.0267	1.0410	1.0555	1.0415
m_x (g)	0.4902	0.5004	0.5006	0.5005	0.5006
m'_y (g)	1.0019	0.9993	0.9989	1.0012	1.0020
m_z (g)	0.5023	0.5013	0.4992	0.5000	0.4988
K_y	1	1	1	1	1
R_y	0.0029	0.0029	0.0029	0.0437	0.0437
K_b	1.0655	1.0729	1.0729	1.0383	1.0365
R_b	0.9050	0.9253	0.9066	0.9321	0.9501
K_x	1	1	1	1	1
R_x	2.2436	2.2436	2.2436	6.8049	6.8049
K'_b	1.0771	1.0711	1.0711	1.0394	1.0315
R'_b	0.9314	0.9403	0.9336	0.9796	0.9817
K_z	1	1	1	1	1
R_z	2.2436	2.2436	2.2436	6.8049	6.8049
$\sum(K_{xi}, R_{xi})$	3.2436	3.2436	3.2436	24.3902	24.3902
$\sum(K_{zi}, R_{zi})$	3.2436	3.2436	3.2436	24.3902	24.3902
B (µmol)	0.0002	0.0002	0.0002	0.0007	0.0007
B (µg)	0.0117	0.0117	0.0117	0.0482	0.0482
D	1	1	1	1	1
W	1	1	1	1	1
$c_{x,i}$ (µmol g^{-1})	0.2271	0.0887	0.0875	0.6797	0.5905
$c_{x,i}$ (mg kg^{-1})	14.4283	5.6364	5.5595	44.4477	38.6106
Recovery (%)		99.9	98.1		100.0

Table 5
Results of the analysis of Ca 317.933/Ca 315.887 ratio

Ca 317.933/Ca 315.887	Standard solutions A_1 and B_1	CCQM soybean	Standard solutions A_2 and B_2	NIST RM 8412
Mean ± standard deviation	1.6454 ± 0.0024 (n = 13)	1.6566 ± 0.0016 (n = 12)	1.6446 ± 0.0015 (n = 5)	1.6531 ± 0.0023 (n = 4)

mass fraction of Ca in the sample was calculated using equation (S11).

3.2.3. Selection of wavelengths

Two wavelengths recommended by the ICP WinLab software for the analysis of Ca were 317.933 nm and 315.887 nm, respectively when a cyclonic spray chamber was used. Two commonly used elements, namely Lutetium (Lu) and Yttrium (Y), were examined for their suitability of being employed as internal standards. One wavelength recommended for Lu was 291.139 nm; and three wavelengths assigned for Y were 371.029, 324.227 and 360.073 nm, respectively. The alternative wavelength of Lu 219.554 was not selected because its sensitivity was not sufficient to provide ample emission intensity for measurement. The concentrations of Lu and Y were approximately 100 and 200 µg L^{-1}, respectively. Preliminary results of the duplicate measurements of Ca in NIST RM 8412 revealed that satisfactory recoveries (100 ± 2%) were obtained based on 8 different combinations of

Table 6
Results of the analyses of CCQM Soybean, NIST SRM 1515, IAEA-359 and NIST RM 8412

	CCQM soybean	NIST SRM 1515	IAEA-359	NIST RM 8412
Mass fraction of Cu (mg kg^{-1})				
(i) CCQM value[a] ± standard deviation (ii) certified value ± expanded uncertainty (iii) reference value ± expanded uncertainty	(i) 14.2 ± 0.76	(ii) 5.64 ± 0.24	(iii) 5.67 ± 0.18	–
Found (mean ± standard deviation)	14.3 ± 0.1 (n = 6)	5.63 ± 0.01 (n = 4)	5.57 ± 0.01 (n = 2)	–
Recovery (%) (mean ± standard deviation)	–	99.8 ± 0.1 (n = 4)	98.2 ± 0.1 (n = 2)	–
Mass fraction of Zn (mg kg^{-1})				
(i) CCQM value[a] ± standard deviation (ii) certified value ± expanded uncertainty (iii) reference value ± expanded uncertainty	(i) 44.8 ± 2.2	–	(iii) 38.6 ± 0.7	–
Found (mean ± standard deviation)	44.6 ± 0.2 (n = 6)	–	38.9 ± 0.2 (n = 4)	–
Recovery (%) (mean ± standard deviation)	–	–	100.7 ± 0.6 (n = 4)	–
Mass fraction of Ca (mg kg^{-1})				
(i) CCQM value[a] ± standard deviation (ii) certified value ± expanded uncertainty (iii) reference value ± expanded uncertainty	(i) 1660 ± 29	–	–	(iii) 2160 ± 80
Found (mean ± standard deviation)	1663 ± 7 (n = 6)	–	–	2156 ± 22 (n = 7)
Recovery (%) (mean ± standard deviation)	–	–	–	99.8 ± 1.0 (n = 7)

[a] CCQM value is the mean of all participants' results given in the final report of CCQM-P64.

analyte wavelengths (Ca 317.933 and Ca 315.887) and internal standard wavelengths (Lu 291.139, Y 371.029, Y 324.227 and Y 360.073). As no significant difference was identified among the results, it could be reasonably ascertained that spectral interferences were negligible in all wavelengths examined. Note that the analyte wavelength Ca 317.933 is recommended by the US Environmental Protection Agency for ICP–AES measurement (USEPA, 1996). Lu 291.139 has been reported as a good choice of internal standard wavelength (Grotti, Magi, & Leardi, 2003). In this connection, we chose Ca 317.933 and Lu 291.139 for subsequent analysis.

3.3. Method validation using CRM and participation in CCQM-P64

3.3.1. Analysis of CRM

Satisfactory recoveries of Cu/Zn were obtained in NIST SRM 1515 (Cu: 99.8 ± 0.1%, $n = 4$; Table 6) and IAEA-359 (Cu: 98.2 ± 0.1%, $n = 2$; Zn: 100.7 ± 0.6%, $n = 4$; Table 6). The success of IDMS relied on the fulfilment of two requirements. First, the blend isotope ratios of $^{63}Cu/^{65}Cu$ and $^{66}Zn/^{67}Zn$ should be in the range of 0.9 to 1. Second, the "approximate match" should be within 10%. During the IDMS analysis, the procedural blanks were found to be about 0.0002 µmol Cu and 0.0007 µmol Zn, respectively. The blank values were negligible when compared with the analyte levels. Recovery of Ca in NIST RM 8412 was quantitative (99.8 ± 1.0%, $n = 7$; Table 6). The ICP–AES result corroborated that the determination of Ca using bracketing technique was accurate and precise.

3.3.2. CCQM-P64

Two major tasks of the CCQM are: (i) to present activities concerning primary methods for measuring the amount of substance; and (ii) to organise a comprehensive set of international comparisons for the purpose of establishing a technical basis for mutual recognition of measurement capabilities among national metrology institutes (NMIs) in the field of chemical measurement (Kaarls & Quinn, 1997). To demonstrate the measurement capability of NMIs, the CCQM inorganic working group organised a pilot study of CCQM-P64 for the determination of essential elements in non-fat soybean powder in 2005. The National Research Center for Certified Reference Materials (Note: It has been united with the National Institute of Metrology People's Republic of China since 2005) acted as the co-ordinating laboratory and was responsible for sample preparation, homogeneity assessment, sample distribution and evaluation of final results. About 20 NMIs participated in the study. As shown in the final report of CCQM-P64 (Ma et al., 2007), participants used various measurement methods (IDMS, ICP–MS, ICP–AES, atomic absorption spectrophotometry, instrumental neutron activation analysis and X-ray fluorescence spectrometry) and digestion techniques (microwave-assisted digestion, acid digestion and dry ashing). We applied the validated methods to the CCQM pilot study. The mass fractions (mean ± standard deviation, $n = 6$) of Cu, Zn and Ca were found to be 14.3 ± 0.1 mg kg^{-1}, 44.6 ± 0.2 mg kg^{-1} and 1663 ± 7 mg kg^{-1}, respectively. The results were in excellent agreement with the mean values of all participants' results (Table 6). It was concluded that our methods, namely ID–ICP–MS with "approximate match" approach and ICP–AES with bracketing technique, showed satisfactory performance in the study. Since complete discussions on participants' results and methodologies used are beyond the scope of this work, readers who are interested may consult the reference cited (Ma et al., 2007).

3.4. Uncertainty estimation

3.4.1. Copper and Zinc

Uncertainty estimation utilised the following parameters: (i) the best available estimate of uncertainty associated with the mea-

surement of Cu/Zn using ID–ICP–MS, u(ID–ICP–MS); and (ii) the best available estimate of overall bias, u(Recovery).

3.4.1.1. Copper and Zinc: Estimation of u(ID–ICP–MS). The approach shown in Example A7 of EURACHEM/CITAC Guide (Ellison, Rosslein, & Williams, 2000) was applied to the quantification of uncertainty components in the measurement of Cu/Zn using ID–ICP–MS. In practice, an Excel spreadsheet based on the numerical method of differentiation as described by Kragten was used (Kragten, 1994). The uncertainty budgets showing all uncertainty sources and their typical values in one of the replicate measurements of the mass fractions of Cu and Zn in the CCQM soybean sample are provided in Tables S5 and S6, respectively. The results of analyses of Cu and Zn were based on the isotope ratios of $^{63}Cu/^{65}Cu$ and $^{66}Zn/^{67}Zn$, respectively. It was imperative to note that the uncertainties due to components K_b, K'_b, R_b and R'_b contributed significant portions of about 90% and 65% to the combined standard uncertainties, $u(c_{x,i})$, in the measurement of Cu and Zn, respectively.

For the measurement of Cu in the sample blend replicate given in Table S5, the signal intensities measured at m/z 63 and 65 were about 380,000 and 420,000 cps, respectively. Also, for the measurement of Zn in the sample blend replicate given in Table S6, the signal intensities measured at m/z 66 and 67 were about 200,000 and 220,000 cps, respectively. The instrumental background was estimated using a blank solution of 1% (v/v) HNO$_3$. The signal intensities recorded for the blank solution were about 300–900 cps at the four analytical masses concerned. The instrumental background was insignificant when compared with the signal intensities of the sample blend replicates. In our work, blank subtraction was not performed on the analyte signal intensity.

Three "additive" corrections for the detector dead time effect (Eq. (2)), the instrumental background (Eq. (3)), and the isobaric interference (Eq. (4)) are usually applied to individual isotope signal intensities.

$$I_{\text{dead time corrected}} = I_{\text{observed}}/(1 - I_{\text{observed}} \cdot \tau) \tag{2}$$

$$I_{\text{instrumental background corrected}} = I_{\text{observed}} - I_{\text{instrumental background}} \tag{3}$$

$$I_{\text{isobaric interference corrected}} = I_{\text{observed}} - I_{\text{isobaric interference}} \tag{4}$$

where $I_{\text{dead time corrected}}$ is the isotope signal intensity corrected for the detector dead time (cps); I_{observed} is the measured isotope signal intensity before correction (cps); τ is detector dead time (s); $I_{\text{instrumental background corrected}}$ is the isotope signal intensity corrected for the instrumental background (cps); $I_{\text{instrumental background}}$ is the measured counting rate for the instrumental background signal (cps); $I_{\text{isobaric interference corrected}}$ is the isotope signal intensity corrected for the isobaric interference (cps); $I_{\text{isobaric interference}}$ is the measured counting rate for the isobaric interference signal (cps).

As suggested in literature, propagating the uncertainties associated with these corrections directly with the repeatability of the measurement of the individual isotope signal intensities leads to an over-estimation of the resulting combined standard uncertainty (Quétel, Prohaska, Nelms, Diemer, & Taylor, 2001). Therefore, "additive" corrections on isotope signal intensities are usually translated into multiplicative unity correction factors on isotope ratios. The isotope ratio R is given in Eq. (5).

$$R = R_0 \cdot \delta_{\text{dead time}} \cdot \delta_{\text{instrumental background}} \cdot \delta_{\text{isobaric interference}} \tag{5}$$

where R_0 is the measured isotope ratio; δ dead time is a unity factor carrying the standard uncertainty (SU) associated with the correction for the detector dead time effect (δ dead time = 1 ± SU dead time); δ instrumental background is a unity factor carrying the standard uncertainty associated with the correction for the instrumental background (δ instrumental background = 1 ± SU instrumental background); δ isobaric interference is a unity factor carrying the

standard uncertainty associated with the correction for the isobaric interference (δ isobaric interference = 1 ± SU isobaric interference); R is the isotope ratio corrected for the aforementioned parameters.

Since the instrumental background was negligible and isobaric interferences were not observed at the four analytical masses concerned, only one "additive" correction for the detector dead time effect was applied to R_0 according to Eq. (6).

$$R = R_0 \cdot \delta_{\text{dead time}} \tag{6}$$

In the present study, the sample and calibration blends were matched within 10%. The detector dead time correction would have an insignificant effect in such a well-matched pair of isotopic blends that had similar isotope signal intensities. As such, no contribution for the detector dead time correction was added in the estimation of the standard uncertainties of the measured isotope ratios.

The standard uncertainty associated with the measurement of the isotope ratio (R_b or R'_b) was calculated as the standard deviation of the mean, which was equal to the standard deviation divided by the square root of the number of repeated measurements ($n = 4$), i.e. $u(R_b \text{ or } R'_b)$ = standard deviation (R_b or R'_b)/$\sqrt{4}$. Similarly, the standard uncertainty associated with the mass bias correction factor was calculated as follows: $u(K_b \text{ or } K'_b)$ = standard deviation (K_b or K'_b)/$\sqrt{4}$. The samples and primary assay standards were assumed to have the Cu and Zn isotopic compositions as provided by the IUPAC (Rosman & Taylor, 1998). The standard uncertainty associated with R_x or R_z was estimated following a procedure described elsewhere (Wolff Briche, Harrington, Catterick, & Fairman, 2001). The isotope ratios (value ± standard uncertainty) of ^{63}Cu/^{65}Cu and ^{66}Zn/^{67}Zn were estimated to be 2.2436 ± 0.0014 and 6.8049 ± 0.0404, respectively. The standard uncertainties for R_y were stated in the respective certificates of Cu and Zn spike solutions. All K_x, K_y and K_z were assumed to be one (Yip et al., 2006).

The primary assay standard solutions of Cu and Zn were prepared gravimetrically through a series of dilution from NIST SRM 3114 and NIST SRM 3168a, respectively. The element amount concentrations of Cu and Zn, c_z, were 0.2268 and 0.6880 µmol g^{-1} and their relative standard uncertainties were estimated to be 0.08% and 0.11%, respectively (Wolff Briche et al., 2001).

For the analysis of the CCQM soybean, a total of six sample replicates were carried out. The mass fractions (mean ± standard deviation, $n = 6$) of Cu and Zn in the sample were 14.3205 ± 0.1038 mg kg^{-1} and 44.6011 ± 0.2059 mg kg^{-1}, respectively. A unity factor, D, was introduced to account for the repeatability in the analysis of sample replicates. The standard uncertainty (expressed in relative term) associated with the measurement of Cu in replicate samples was calculated as follows: $u(D)$ = (standard deviation/$\sqrt{n}$)/mean = (0.1038/$\sqrt{6}$)/(14.3205) = 0.0030. For the measurement of Zn, $u(D)$ = (0.2059/$\sqrt{6}$)/(44.6011) = 0.0019. Since only two procedural blanks were carried out in the same analytical run, the best estimate of the standard uncertainty was that $u(B)$ = standard deviation (B)/$\sqrt{2}$.

Following the Kragten's approach, the values of u(ID–ICP–MS) for the measurement of mass fractions of Cu and Zn in the CCQM soybean sample were estimated to be 0.0123 (Table S5) and 0.0173 (Table S6), respectively.

3.4.1.2. Copper and Zinc: Estimation of u(Recovery). Overall bias was estimated by the repeated analysis of IAEA-359. For the measurement of Cu, a total of 6 portions were analyzed. Each portion was gone through the entire analytical procedure. The results are shown in Table S7. Recovery and its uncertainty were calculated using Eqs. (7) and (8), respectively. The values of Recovery and u(Recovery) were found to be 0.9787 and 0.0157, respectively. Similarly, for the measurement of Zn, a total of 8 portions were examined. The results are shown in Table S8. The values of Recov-

ery and u(Recovery) were found to be 1.0068 and 0.0094, respectively.

$$\text{Recovery} = \frac{C_{\text{obs}}}{C_{\text{RM}}} \tag{7}$$

$$u(\text{Recovery}) = \text{Recovery} \times \sqrt{\left(\frac{s_{\text{obs}}/\sqrt{n}}{C_{\text{obs}}}\right)^2 + \left(\frac{u(C_{\text{RM}})}{C_{\text{RM}}}\right)^2} \tag{8}$$

where C_{obs} is the mean of the replicate analyses of the reference material (mg kg^{-1}); C_{RM} is the value assigned for the reference material (mg kg^{-1}); s_{obs} is the standard deviation of the results from the replicate analyses of the reference material (mg kg^{-1}); n is the number of replicates; $u(C_{\text{RM}})$ is the standard uncertainty in the value assigned for the reference material (mg kg^{-1}).

A significance test was carried out to determine whether the recovery was significantly different from 1. The test statistic t was calculated using Eq. (9). For the measurement of Cu and Zn, the values of t were found to be 1.4 and 0.74, respectively, which were less than the coverage factor k of 2 for calculating the expanded uncertainty. As the recoveries were not significantly different from one in both cases, no correction was made to analytical results.

$$t = \frac{|1 - \text{Recovery}|}{u(\text{Recovery})} \tag{9}$$

3.4.1.3. Copper and Zinc: Combined standard uncertainty. Finally, the relative standard uncertainties relating to ID–ICP–MS and recovery were combined. The relative expanded uncertainties ($k = 2$) for the measurement of mass fractions of Cu and Zn in the CCQM soybean sample were estimated to be ±4.0% (Table S9) and ±3.9% (Table S10), respectively.

3.4.2. Calcium

To quantify uncertainty components relating to the measurement of Ca, the approach given in Example A4 of EURACHEM/CI-TAC Guide was followed. Uncertainty estimation utilised the three parameters: (i) the best available estimate of overall precision, u(Precision); (ii) the best available estimate of overall bias, u(Recovery); and (iii) other sources of uncertainty.

3.4.2.1. Calcium: Estimation of u(Precision). The standard deviations of the replicate measurements of the CCQM soybean sample over a month are shown in Table S11. The relative standard deviations (RSD) were of the same order of magnitude and were pooled to obtain the estimate of u(Precision) using Eq. (10). u(Precision) was therefore 0.0048 as a relative standard deviation.

$$\text{RSD}_{\text{pool}} = \sqrt{\frac{\sum((n_i - 1) \times \text{RSD}_i^2)}{\sum(n_i - 1)}} \tag{10}$$

3.4.2.2. Calcium: Estimation of u(Recovery). Overall bias was estimated by the repeated analysis of NIST RM 8412, which contains a level of Ca similar to the CCQM soybean sample. A total of 13 portions were analyzed. The results are shown in Table S12. The values of Recovery and u(Recovery) were found to be 0.9996 and 0.0187, respectively.

A significance test was performed and the value of t was found to be 0.02, which was less than the coverage factor k of 2 for calculating the expanded uncertainty. Again, as the recovery was not significantly different from one, no correction was made.

3.4.2.3. Calcium: Estimation of other sources of uncertainty. Other sources of uncertainty associated with volumetric measurements were taken into consideration. It was noteworthy that 100 mL vol-

umetric flasks used in the preparation of standard solutions of Ca were of Class A and were verified before use (BS EN ISO 1042, 2000). The acceptance limit for Class A narrow-necked volumetric flasks was 100 ± 0.1 mL. In order to ensure that the volumes of stock standard solutions delivered (i.e. 0.100–0.220 mL as shown in Table S4) were precise and accurate, the electronic pipette was calibrated at two settings of nominal volumes of 0.125 mL and 0.250 mL, respectively (BS 700-1, 1982). Calibration was performed at the temperature of 21.5 °C, which was within the working temperature range of 18–25 °C as suggested by the manufacturer of the pipette. Precision (RSD%) was found to be less than the acceptance criterion of 0.15% for ten repeated measurements of water delivered at each setting. Deviations from the two nominal volumes were found to be less than the acceptance criteria of ± 1 μL and ± 2 μL, respectively.

3.4.2.4. Calcium: Combined standard uncertainty. The uncertainties associated with the purity of Ca standard solution, the weighing balance, the electronic pipette, and volumetric flasks were negligible because their values (in relative terms) were far less than 1/5 of u(Precision) or of u(Recovery). The relative standard uncertainties relating to precision and recovery were combined. The relative expanded uncertainty ($k = 2$) for the measurement of mass fraction of Ca in the CCQM soybean sample was estimated to be ±3.9% (Table S13).

4. Conclusion

We have shown that the two validated methods, namely (i) ID–ICP–MS with "approximate match" approach and (ii) ICP–AES with bracketing technique, are considered suitable for the measurement of mass fractions of Cu, Zn and Ca, respectively in soybeans. The measurement results are traceable to the SI. As far as we know, there is no reference material made of soybeans with certified values of trace elements available in the market. Satisfactory results achieved in the CCQM-P64 pilot study have demonstrated the suitability of the methods for the certification measurement of the three elements in soybeans.

Acknowledgements

Appendix A. Supplementary material

Supplementary data (including experimental details, equations (S1)–(S11), Tables S1–S13) associated with this article can be found, in the online version, at doi:10.1016/j.foodchem.2008.06.067.

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