Abstract:
The concentrations of arsenic (As), cobalt (Co), and copper (Cu) were determined in Thai local rice cultivated in different areas. The analytical method was validated by the rice flour certified reference material (SRM 1568b) in order to verify the precision and accuracy for the determination of As, Co, and Cu in rice samples using inductively coupled plasma mass spectrometry (ICP-MS) with internal standard method. The recoveries of As, Co, and Cu were 97.4±3.3, 101.4±6.8, 91.3±2.4 %, respectively. The precision of the method in terms of %RSD were less than 6.8. The proposed method gave linear calibrations with correlation coefficients greater than 0.999. Limits of detection (LODs) and limits of quantitation (LOQs) were 0.023-0.078 and 0.065-0.161 μg/L, respectively. The concentrations of As, Co, and Cu in Thai local rice samples were obtained in the range of 0.045-0.284, 0.011-0.130, 0.781-2.419 mg/kg, respectively.

1. Introduction
Rice (Oryza sativa) is a staple food for more than half of the world’s population and is the most important crop of Thailand. Rice is also a stable food for Thai population. Concerning about of heavy metals in rice is an important because they accumulate continuously in paddy fields and consequently affect animal and humans via food chain process. The contaminations of heavy metals in rice sample is mainly caused from the use of chemical fertilizer, pesticides, and contaminated irrigation water which directly affect agricultural crops through cultivation. Heavy metals are generally toxic at low concentration, non-biodegradable and bio-accumulate in the body for a long period exposure. Arsenic is directly toxic and has been registered as carcinogen by International Agency for Research on Cancer (IARC). Some studies have reported the evidences of carcinogenic risk through inhalation, dermal contact and ingestion and consequently resulting in lung, liver, skin and bladder cancer in humans. Cobalt (Co) and copper (Cu) are essential nutrients for plants and animals. Since they have a variety of biochemical functions in organisms. However, they can be toxic and harmful to human health at a high concentration.

Several studies have reported the determination of heavy metal contamination in rice samples by various analytical techniques. Parengam et al. used instrumental neutron activation analysis (INAA) and graphite furnace atomic absorption spectroscopy (GFAAS) techniques to determine the concentration of Al, As, Br, Ca, Cr, Cu, Fe, Hg, I, K, Mg, Mn, Mo, P, Se, Sn, V and Zn in cereals (rice) and legumes. Although sample preparation step for INAA is not necessary but it requires a nuclear reactor which is not available in few laboratories. Rahman et al. used ICP-MS to determine the concentrations of cadmium (Cd), cobalt (Co), chromium (Cr), copper (Cu), manganese (Mn), nickel (Ni), lead (Pb), and zinc (Zn) in rice.

According to the literature, ICP-MS technique is a powerful technique, rapid and high sensitive multi-elemental analysis. Microwave digestion has many attractive features such as simplicity, rapidity, good repeatability, reliable and minimal reagent
consumption when compares with traditional digestion. Therefore, the aim of this study was to evaluate the contamination of three heavy metals: arsenic, cobalt and copper in Thai local rice using ICP-MS with microwave digestion as method for sample preparation. Method validation was evaluated by reference material (SRM 1568b rice flour).

2. Materials and Methods

2.1 Sample preparation

Thai local rice samples were collected in paddy fields from Kalasin and Surin. The polished rice was ground to be fine powder, sieved through a 0.70 mm-mesh, oven-dried for two days at 60 °C and finally kept in desiccator prior to analysis by ICP-MS.

2.2 Sample Digestion

A 0.8 g powdered rice sample was weighted into a polytetrafluoroethylene (PTFE) digestion vessels and 10 mL HNO₃ was then added. The sample was digested in a Mars™ 6 Xpress microwave digestion system (CEM, NC, USA). The digestion conditions used for the microwave digestion were 15-min ramp and 30-min hold at 950 W. After cooling, the digested solution was evaporated to dryness in order to remove residual concentrated nitric acid. Finally, the sample was diluted with 10 mL of 2% (v/v) HNO₃ and then filtered through a 0.45 µm syringe filter. For method validation, 0.8 g of the certified reference material NIST 1568b rice flour was used to verify the accuracy of the method. The limits of detection (LODs) and limits of quantitation (LOQs) are defined as the concentration equivalent to the signal of 3 and 10 times the standard deviation above the signal of reagent blank, followed in Eurachem.⁹

2.3 ICP-MS analysis

All heavy metals were determined using an ICP-MS (Agilent 7900, Agilent Scientific Technology Ltd., USA). All samples and standards were added with ¹¹⁵In as internal standard (5 µg L⁻¹) and measured using He as collision gas (m/z=⁷⁵As, ⁵⁹Co and ⁶³Cu). The polyatomic interferences can be eliminated in a collision cell. Each solution was measured in triplicate. The detailed instrumentation and operating conditions are shown in Table 1.

<table>
<thead>
<tr>
<th>Isotope monitored (m/z)</th>
<th>As ⁷⁵, Co ⁵⁹, Cu ⁶³</th>
</tr>
</thead>
</table>

3. Results & Discussion

The results of method validation of ICP-MS technique for heavy metals analysis in Thai local rice samples are shown in Table 2. Good linearity was obtained with the correlation coefficient (R²) greater than 0.999 for each element. The limits of detection (LOD) were 0.078, 0.023 and 0.024 mg kg⁻¹ for As, Co and Cu, respectively. Limits of quantitation (LOQs) were in the range of 0.065 to 0.161 mg kg⁻¹. The relative standard deviations (RSD) of the measurements of heavy metals ranged from 2.65% to 6.67% which was satisfied for the purpose of this work. The recovery of the analytical method was evaluated by SRM1568b and found to be 97.4, 101.4 and 91.3% for As, Co and Cu, respectively. The certified values of As, Co and Cu in SRM1568b are 0.285, 0.0177 and 2.35, respectively. As, Co and Cu concentrations were not different between the measured values and the certified values at 95% confidence level with p-values of 0.308, 0.761 and 0.107 for As, Co and Cu, respectively.

The concentrations of As, Co and Cu found in Thai local rice is presented in Fig. 1 as the different symbols. The ranges of the
studied elements, maximum, minimum, and median found in the Thai local rice samples are also shown in Table 3. The arsenic content of Thai local rice samples ranged from 0.045 to 0.284 mg kg\(^{-1}\) with a mean of 0.187 mg kg\(^{-1}\). The presence of arsenic may be the long-term use of arsenic-based pesticides, herbicides and fungicides. Moreover, As presented in rice causes from the use of contaminated water of irrigation. These are possible transferring from water to plant. A limit for arsenic in rice has not been established by CODEX.\(^7,10-11\)

Cobalt concentration ranged from 0.011 to 0.130 mg kg\(^{-1}\) with an average value of 0.038 mg kg\(^{-1}\). Cobalt is a constituent of some metalloproteins and vitamin B12. There is a mechanism to support in the normal functioning of the brain and nervous system, and for the formation of blood.\(^10\)

The Cu concentrations in rice samples were found range of 0.781 to 4.670 mg kg\(^{-1}\) with an average concentration of 2.419 mg kg\(^{-1}\). There is no evidence of standard maximum limit for Cu in rice. WHO recommended a dietary reference adequate

Table 2 The results of the validated method verified with SRM1568b (rice flours, NIST). (Give linear equation of calibration curves)

<table>
<thead>
<tr>
<th>Element</th>
<th>Working range ((\mu g\ L^{-1}))</th>
<th>(R^2)</th>
<th>LODs ((\mu g\ L^{-1}))</th>
<th>LOQs ((\mu g\ L^{-1}))</th>
<th>Recovery (%)</th>
<th>RSD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>As</td>
<td>0.025-2.5</td>
<td>0.9993</td>
<td>0.078</td>
<td>0.161</td>
<td>97.4</td>
<td>3.39</td>
</tr>
<tr>
<td>Co</td>
<td>0.1-5.0</td>
<td>0.9998</td>
<td>0.023</td>
<td>0.065</td>
<td>101.4</td>
<td>6.67</td>
</tr>
<tr>
<td>Cu</td>
<td>0.1-2.5</td>
<td>0.9999</td>
<td>0.024</td>
<td>0.066</td>
<td>91.3</td>
<td>2.65</td>
</tr>
</tbody>
</table>

Table 3 Median, maximum, minimum and mean of concentrations (mg kg\(^{-1}\)) of arsenic, cobalt and copper in Thai local rice samples (n=18).

<table>
<thead>
<tr>
<th>Element</th>
<th>Max</th>
<th>Min</th>
<th>Mean</th>
<th>Median</th>
</tr>
</thead>
<tbody>
<tr>
<td>As</td>
<td>0.284</td>
<td>0.045</td>
<td>0.187</td>
<td>0.184</td>
</tr>
<tr>
<td>Co</td>
<td>0.130</td>
<td>0.011</td>
<td>0.038</td>
<td>0.026</td>
</tr>
<tr>
<td>Cu</td>
<td>4.670</td>
<td>0.781</td>
<td>2.419</td>
<td>2.381</td>
</tr>
</tbody>
</table>

Fig. 1. The variation of As, Co and Cu found in Thai local rice.
intake (AI) of 0.900 mg day\(^{-1}\) and a tolerable upper intake for males and females (aged 19–70 years) of 10 mg day\(^{-1}\) for Cu.\(^9\) It may cause liver damage, irritation of the nose, mouth, and eyes, headaches, stomachaches, dizziness, acute gastrointestinal effects and insomnia if Cu exposure exceeds the recommended limit.\(^{12}\)

4. Conclusion

The determination of As, Co and Cd in Thai local rice samples by ICP-MS after digestion by microwave digestion was studied. The method validation has proved to be effective in the quantification of As, Co and Cu in rice samples, with % recovery higher than 91. The relative standard deviations (RSD) were below 6.8%. Limits of detection (LOD) of 0.078, 0.023 and 0.024 mg kg\(^{-1}\) for As, Co and Cu were obtained. The present study will be a good source of information for estimation of the total dietary intake and health risk assessment of the heavy metals from Thai local rice consumption for Thai consumers.

Acknowledgements

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References


11. Antoine, J.M.R.; Fung, LA. H.; Grant, C.N.; Dennis, H.T.; Lalor, G.C. Dietary