

**Optimization of extraction conditions and validation of the method for
determination of arsenic species in carrageenan-producing seaweed,
Kappaphycus spp., Using HPLC-ICP-MS**

ABSTRACT

Seaweeds are known for their nutritional benefits; however, they contain one of the highest levels of arsenic content. Arsenic species are found in various oxidation states in the environment, where inorganic arsenic is more toxic than organic ones. This study describes the optimization of extraction conditions for the determination of arsenic species, namely, arsenate [As(V)], arsenite, [As(III)], arsenobetaine (AsB), dimethylarsenic acid (DMA), and monomethylarsenic acid (MMA) in *Kappaphycus alvarezii* (*K. alvarezii*), a carrageenan-producing red seaweed, using high-performance liquid chromatography inductively coupled plasma-mass spectrometry (HPLC-ICP-MS). A series of nitric acid solutions (0 to 2.0%) as extraction solutions and various extraction times (0 to 240 min) were investigated with heating at 90 °C. The proposed method was validated for matrix effects, linearity and working range, the limit of detection (LOD), the limit of quantification (LOQ), repeatability, within-lab reproducibility, and trueness using certified reference material (CRM, NMIJ 7405-a) and spiking with arsenic species. Among the nitric acid concentration tested, the concentration of 0.2% nitric acid gave recovery values of 86.8 to 94.2%, which complied with the European guidelines 2002/657/EC. Arsenic species were recovered at the maximum level from *K. alvarezii* at the extraction time of 60 min. The proposed method measured the As(V) concentration in CRM as $9.7 \pm 0.3 \text{ mg kg}^{-1}$ and therefore was compliant with the certified level of $10.1 \pm 0.5 \text{ mg kg}^{-1}$. The measurement of trueness for the fortified seaweed was within the acceptable limits, with recoveries between 87.8 and 107.4% for all five arsenic species. The proposed method demonstrated accuracy by complying with the provisions stated in the guidelines for validation performance characteristics. The efficacy of this method was further validated for the determination of arsenic species in *K. alvarezii* varieties.