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**Two-dimensional (Weak Anion Exchange Chromatography-Gel Electrophoresis) Separations Coupling to Inductively Coupled Plasma Mass Spectrometry Strategy for Analysis of Metalloproteins**

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**Abstract**

A two-dimensional (2-D, weak anion exchange chromatography-gel electrophoresis, WAX-GE) separations coupling to inductively coupled plasma mass spectrometry (ICP-MS) strategy was developed for efficient tracking of metalloproteins. Samples were fractionized with the primary dimension (WAX) and the collected fractions were subsequently subjected to the secondary dimension (GE) and finally detected with ICP-MS. The metalloproteins of interest from the secondary dimension were online split and collected for further manipulation. The molecular weight of metalloprotein was calibrated with a home-made protein marker including six iodine-labelled proteins with molecular weights ranging from 14 kDa to 77 kDa. The developed 2-D system is of higher separation resolution as compared to one-dimensional (1-D) system. Metalloproteins in *Escherichia coli* were further examined for validation of the method preformation. Several known or unknown metal-associated proteins were identified, evidencing the feasibility of the proposed method. Taken together, we

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