

ORIGINAL PAPER

Analytical protocol for investigation of zinc speciation in plant tissue[‡]

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A specific procedure is proposed for investigating the chemical speciation of zinc (Zn) in plant tissues, viz., the extraction of Zn compounds from *Plantago lanceolata* L. followed by the chromatographic separation and inductively coupled plasma mass spectrometry (ICP-MS) identification of these compounds. In order to separate the Zn compounds, both size-exclusion (SEC) and ion-exchange liquid chromatography (IC) were used in direct sequential and reverse sequential modes. In the direct sequential mode, the entire extract undergoes SEC separation and then the individual fractions are injected onto the ion-exchange column. The molecular size distribution is evaluated by SEC coupled on-line to the UV detector. In the reverse sequential mode, the entire extract undergoes the ion-exchange chromatographic separation and then the individual fractions are injected onto the size-exclusion column. The identification of Zn incorporated into the compounds is further performed using ICP-MS. This procedure is particularly useful in speciation studies when identification of the individual components of the element is problematic due to the lack of suitable standard substances, as is the case for Zn compounds. The proposed procedure facilitates assignment of the signals to the individual components of the fractions for both types of chromatography, thus rendering the chemical speciation of Zn possible when the lack of suitable standard substances impedes the identification of individual components.

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Introduction

Zinc (Zn) is deemed a microelement essential to living organisms, as it is known to play the role of cofactor in more than 200 enzymes and exhibits a structural role in many proteins. Despite the low Zn content in many organs, Zn metallo-enzymes are widespread throughout the body, playing a crucial role in many physiological processes (Baranowska-Morek, 2003). Nowadays, appropriate doses of a digestible form of Zn are often not achieved, hence knowledge of the Zn metabolism in plants is important in devel-

oping functional foods which would supplement this element in the human diet (Alloway, 2008).

Zn can also be harmful when entering the environment as industrial pollution, resulting in the incidence of various adaptive responses in plants growing in polluted areas. Metallophytes such as *Plantago lanceolata* L. afford a specific example of plants adapted to the harmful conditions of mining waste sites; these not only accumulate metals (e.g. Zn) but also develop a specific mechanism protecting the plant from harmful environmental influences (Wierzbicka et al., 2004).

For understanding of the mechanism enabling

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