FACTORIAL DESIGN FOR METHOD DEVELOPMENT AIMING AT SELENIUM DETERMINATION IN BROCCOLI PARTS BY GF AAS

Daiane Placido Torres[a,b], Š. Mechora[c], R. E. Bruns[b]

[a] *Brazilian Agricultural Research Corporation (EMBRAPA), Embrapa Clima Temperado. Pelotas - RS – Brazil.*

[b] *University of Campinas (Unicamp), Institute of Chemistry, Campinas, São Paulo, Brazil.*

[c] *University of Maribor, Faculty of Natural Sciences and Mathematics, Maribor, Slovenia.*

daiane.torres@embrapa.br

Keywords: Selenium; broccoli; factorial design

Introduction

Selenium (Se) is essential for the activity of 25–30 genetically unique enzymes (selenoenzymes) (Torres et al., 2014). It is one of the elements that plays the most important role in human and animal health. Meanwhile, the availability of selenium for plants depends on soil properties, including pH, salinity and content of $CaCO_3$ (Kabata-Pendias, 2001). Additionally, most methods available for analyte determinations focus on analyzing the edible part of the plants, giving little or no attention to the other parts of the plants, which can also accumulate elements in a situation of exposure, and therefore, are very important concerning an analytical method as well. In this sense, an analytical method was developed in order to measure selenium in different parts of broccoli (stems, leaves, roots, curd), as the plants were previously exposed to selenium species. The technique chosen to measure Se was GF AAS, considering its high sensitivity, and the samples were prepared as slurries in nitric acid, which greatly facilitates the sample preparation process. (Torres et al., 2014; Kabata-Pendias, 2001).

Methods

All measurements were carried out using atomic absorption spectrometer with longitudinally-heated graphite atomizer with Zeeman-effect background correction. Samples were freeze dried before slurry preparation. The employed conditions for Se determination were: 20 µL of the sample or standard solution; 5 µL of the mixture Pd-Mg as chemical modifiers in solution. The pyrolysis and atomization temperatures were optimized and the adopted conditions were 600/2100 ºC. The calibration standard solutions were prepared in 6.67% v/v nitric acid medium and the calibration ranges were from 10.0 to 100.0 µg L^{-1}. The limit of detection and limit of quantification, calculated as three times the standard deviation of 10 measurements of the blank solution and then divided by the slope of the calibration curve, in the sample, were 0.06 µg g^{-1} and 0.21 µg g^{-1}, respectively. The method optimization was performed by using 2^{5-2} factorial design, and using the combinations of the experimental variables, such as mass of sample (0.2000 or 0.2500 g), nitric acid volume (1 or 1.2 ml), sulfuric acid volume (0 or 0.2 ml), ultrasound time (60 or 120 min), and heating of ultrasound bath (none or ~80 °C), the method could be optimized. The sample volume was made up to 15.0 mL with deionized water.

Results

Fractional factorial design is a valuable tool to evaluate the parameters involved in an analytical method, which can, for example, simplify the analytical procedure, reduce the time of a specific step or save reagents. The design was performed with duplicate samples. This choice provides enough degrees of freedom to determine experimental error and evaluate of the design results via Pareto chart. Figure 1 shows the Pareto Charts obtained from this study. From this graph it is possible to observe that none of the evaluated factors

presented significant influence, meaning that we can use the level of the evaluated factors to our best convenience. That means, a sample mass of 0.2500 grams (for higher sensitivity), 1 ml of nitric acid (less reagent), exclusion of sulfuric acid, 60 minutes of ultrasound under no heating. Regarding the two last factors, although there was no statistical significance for the tested levels, the method best benefit from longer times in the ultrasound bath under heating, which produces a clearer slurry, a desired quality when dealing with slurry technique. Then, we adopted 120 minutes under heated ultrasound bath. From the evaluation of stems, leaves, curd, pupae and roots of broccoli plants exposed to Se, the concentration of Se varied from below the LOQ to 60 µg g^{-1} (leaves of broccoli exposed to 500 µg of Se(VI)).

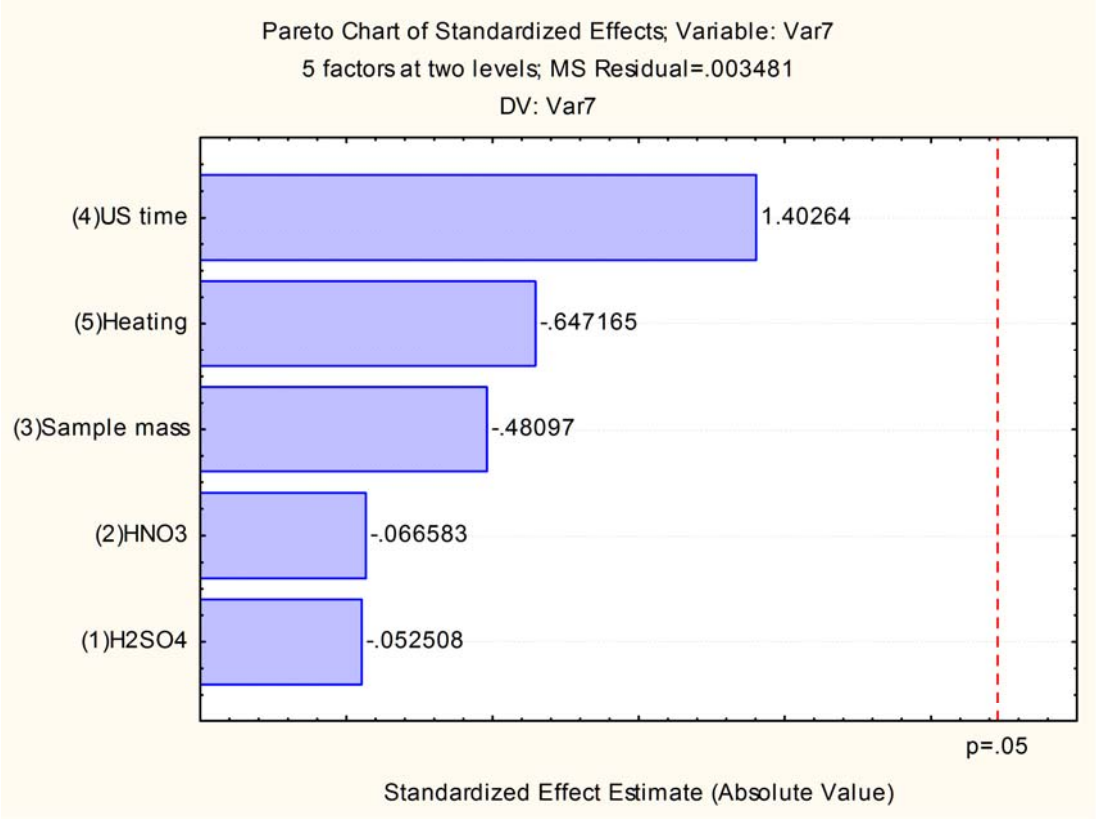

Figure 1. 2^{5-2} factorial design for sample preparation optimization as slurries of broccoli parts for the determination of Se by GF AAS. Sample 15P 25/28 RL (1.26 ug/g of Se).

Conclusion

Acidic slurries of broccoli plant parts obtained after ultrasound exposure has shown to be a reliable and simple way for sample preparation aiming the measurement of Se by GF AAS, which was greatly facilitated by applying a 2^{5-2} fractional factorial design in order to optimize the levels of the method parameters. In this context, 0.2500 grams of the freeze-dried sample was weighed directly in the PP tubes. After that, 1.0 mL of nitric acid was added. Samples were submitted to an ultrasound step for 2 h at 80 °C, and then the volume was made up to 15.0 mL with deionized water.

References

Torres, D.P.; Cadore, S.; Raab, A.; Feldmann, J.; Krupp, E.M. (2014). *J. Anal. At. Spectrom.*, *29*, 1273-1281.

Kabata-Pendias, A. (2001). Trace elements in soil and plants. Third ed., CRC Press, Boca Raton, Florida, USA, pp. 241-252.