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Original Article

RAPID DETERMINATION OF MANGANESE IN FRESH PARSLEY LEAVES AND ROOTS USING CAPILLARY ELECTROPHORESIS METHOD

Błażej Grodner[1*], Małgorzata Jelińska[2]

[1] *Chair and Department of Biochemistry and Pharmacogenomics, Medical University of Warsaw, 1 Banacha Str., 02-097 Warsaw, Poland.*

[2] *Chair and Department of Bromatology, Medical University of Warsaw, 1 Banacha Str., 02-097 Warsaw, Poland.*

* Correspondence, e-mail: blazej.grodner@wum.edu.pl

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ABSTRACT

This is the first work presenting a simple, rapid and sensitive method using capillary electrophoresis (CE) for the direct determination of manganese (II) in fresh parsley leaves and roots. The developed method has been optimized and validated. Optimum separation of Mn^{2+}, by measuring at 214 nm, was obtained on a 30 cm × 75 µm capillary using a 50 mM phosphate running buffer (pH 3.0), capillary temperature 25°C, voltage 10 kV, and hydrodynamic injection. Good results were obtained for different aspects including stability of the solutions, linearity, and precision. Detection and quantification limits for Mn^{2+} were reached at 0.97 and 2.94 µg/mL, respectively. The sample preparation procedure is very simple and not time-consuming. This method has been successfully used to measure the concentration of Mn^{2+} in order to determine the content of this ion in parsley leaves and roots. The speed of obtaining the result of the analysis was only 3 minutes.

KEYWORDS: Capillary electrophoresis, Manganese, Parsley, Food.

1. Introduction

Manganese has many important functions in the body. It is responsible not only for the work of our brain, but also affects the nervous and skeletal systems. Its deficiency heralds many serious health problems [1-4]. Manganese is one of the many elements that are essential to life due to its role in the regulation of enzymatic processes related to the metabolism of proteins and fats [5]. It protects against the appearance of osteoporosis and affects the skeletal system. It is responsible for the synthesis of erythrocytes (red blood cells). It also participates in the process of proper digestion as well as the absorption of proteins, fats and carbohydrates. Manganese is also responsible for the proper functioning of the brain and is involved in the metabolism of cholesterol and the production of thyroxine [6].

The deficiency of manganese is very dangerous for the body. It can lead to a delay in physical development, decreased fertility, bone defects, and also disorders of the nervous system. Some doctors say that a deficiency of this element increases the risk of epilepsy [7].

Manganese may be neurotoxic. Its excess is manifested by muscle pain, memory problems, depression, thyroid and liver disorders, fatigue and trembling limbs. Manganese poisoning impairs the functioning of the nervous system and brain, which contributes to the development of symptoms that resemble Parkinson's disease [8-12].

The above clearly shows that manganese is an extremely important element whose deficiency and excess can lead to a number of health consequences. We should also be aware that this element is supplied to our bodies with many food products. Therefore, it seems important to investigate the manganese content in the products we most often consume. In this paper, we present the development of a method of capillary electrophoresis (CE) for the determination of manganese (II) ions in plant products. For the study we selected fresh parsley leaves and roots.

There are, of course, many methods of determining manganese ions [13-17]. These papers present various methods for the determination of manganese ions. Some of these works describe the possibility of using capillary electrophoresis for the determination of manganese ions in a lithium ion battery [13]. The determination of manganese ions in biological material (blood) was successfully carried out using one of the most interesting HPLC methods (SEC-HPLC-ICP-AES) [14]. The HPLC method (RP-HPLC) was also used to determine the content of manganese ions in drinking water [15]. One of

the first and oldest methods allowing for the determination of manganese ions with very good sensitivity was the colorimetric method based on the color reaction of formaldoxime and ammonia with manganese ions [16]. A modernized variant of this method was used in the spectrophotometric method for the determination of manganese ions [17].

This paper presents a very quick CE method of manganese ions direct determination in fresh plant material.

2. Materials and methods

2.1. Chemicals and reagents.

The sodium diphosphate, phosphoric acid, manganese chloride, calcium chloride, magnesium chloride, copper chloride were purchased from Sigma-Aldrich (Darmstadt, Germany), deionized water and n-hexane from Sigma-Aldrich (Steinheim, Germany).

2.2. Instrumentation

A Capillary Electrophoresis Beckman Coulter P/ACE MDQ system, equipped with an autosampler and a UV/Visible detector, was used. All the parameters of the CE were controlled by Karat software version 32. An eCAP fused-silica capillary (30 cm total length, 20 cm effective length, 75 µm id, 375 µm od) was used.

2.3. CE conditions

The capillary was thermostated at 25°C. Detection was performed in the UV at 214 nm. The results were processed using a Karat software version 32. Hydrodynamic sample injection under the pressure of 2 psi was performed in 5 seconds. Prior to work, the capillary was successively washed with a 0.1 M NaOH solution for 5 min, then with water for 5 min and with a background electrolyte solution for 10 min; between the analyses it was washed with a background electrolyte solution (50 mM sodium diphosphate adjusted to pH = 3.0 with phosphoric acid) for 3 min. The separation process was carried out at voltage of 10 kV.

2.4. Preparation of stock and working standard solutions

The primary standard stock solution of manganese (II) chloride with a concentration of 10.0 mg/mL, in terms of the amount of Mn^{2+} ions was prepared by dissolving 226.8 mg of manganese (II) chloride in 10 mL of deionized water and then diluted 100 times to a concentration of 1000 µg/mL Mn^{2+} and stored in 3 °C. This solution was further diluted with deionized water every day to obtain working standard solutions of appropriate concentrations form 1.0 µg/mL to 300.0 µg/mL. To determine the calibration curve, 6 standard solutions were prepared with the following concentrations: 1.0, 10.0, 20.0, 50.0, 100.0 and 300.0 µg/mL. Each concentration of the analyte was evaluated based on the peak area with respect to a quantitative calibration.

2.5. Sample preparation

In the purification of samples, 10 g of parsley leaves and 10 g of roots have been ground to homogeneous masses in a high-speed electric grinder. Then, 1 g of each preparation was taken from this, and 1 mL deionized water was added. The whole was vortexed for 5 minutes and then heated in a water bath at 70°C for 5 minutes, after which it was vortexed again for 5 minutes. Then the entire sample was centrifuged (5 min, 1000 x g) and the supernatant was filtered through a filter (0.45 µm) and transferred to a second tube. Then the samples were introduced into the capillary and subjected to the separation process.

3. Method validation

The developed method was validated for linearity, specificity, precision, accuracy, extraction recoveries, and limits of detection (LODs) and quantification (LOQs), according to the International Council on Harmonization (ICH) guidelines [18] and guidelines on bioanalytical method validation [19].

4. Results

To determine the best conditions for manganese (II) determination, the following parameters were tested with the developed CE method: analytical wavelength (λ), buffer (sodium diphosphate (50 mM) adjusted to pH 3.0 with phosphoric acid) (C), voltage (kV), and temperature (T). Each of the parameters was tested in the order of their increasing values and presented in the form of the dependence of the detection level and migration rate on the value of five measurement points (Figure 1). The measurement points presented the following parameter values:

(A) λ=300 nm, buffer C=200 mM, kV=25, T=10 °C

(B) λ=280 nm, buffer C=150 mM, kV=20, T=15 °C

(C) λ=254 nm, buffer C=100 mM, kV=15, T=20 °C

(D) λ=214 nm, buffer C=50 mM, kV=10, T=25 °C

(E) λ=200 nm, buffer C=25 mM, kV=5, T=30 °C

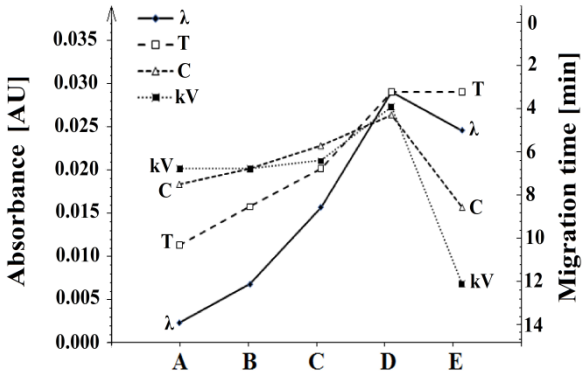

Figure 1. Optimization parameters of the method.

Among the five tested buffer concentrations, the shortest migration time and the highest level of detection were obtained at 50 mM of sodium diphosphate pH = 3.0. The best wavelength with the highest detection level was λ = 214 nm. The study of the influence of temperature and voltage changes on the level of detection and migration rate of the determined compound showed that

the best temperature was 25 ºC and the voltage of 10 kV (Figure 1(D)). The specificity of the method was demonstrated by the lack of effect of other substances present in the investigated sample on the test/determined substance (Mn^{2+}) (Figure 2). In addition, the specificity of the method with respect to Mn^{2+} ions was determined in the presence of standards of other divalent ions, such as Ca^{2+}, Mg^{2+} and Cu^{2+}, subjected to the separation process (Figure 2B). For the ion migrating between Mg and Cu ions, a specific color reaction was performed [16], additionally confirming the presence of Mn^{2+} ions in the samples. We additionally confirmed the specificity by adding a specified amount of the standard (Mn^{2+}) to the plant preparations (Figure 2C and 2D).

All calibration curves showed good linear regression (r2 = 0.999). The LOD (S/N = 3) for Mn^{2+} ions was 0.97 µg/mL, and the LOQs (S/N = 10) for Mn^{2+} ions was 2.94 µg/mL (Table 1). The recoveries of the concerned analytes from aqueous solutions were found to be in a satisfactory range of 99.3–100.0 % (Table 2).

Table 1. Regression equation, limits of detection (LOD) and quantification (LOQ) for Mn^{2+} ions (n=6).

Analyte	Linearity range	R^2	RSD	LOD	LOQ	Regression equation	Standard Deviation	
	µg/mL		(%)	µg/mL	µg/mL		Slope	Intercept
Mn^{2+}	1.00 - 300	0.999	2.14	0.97	2.94	y=1.002x-0.172	±0.0006	±0.0072

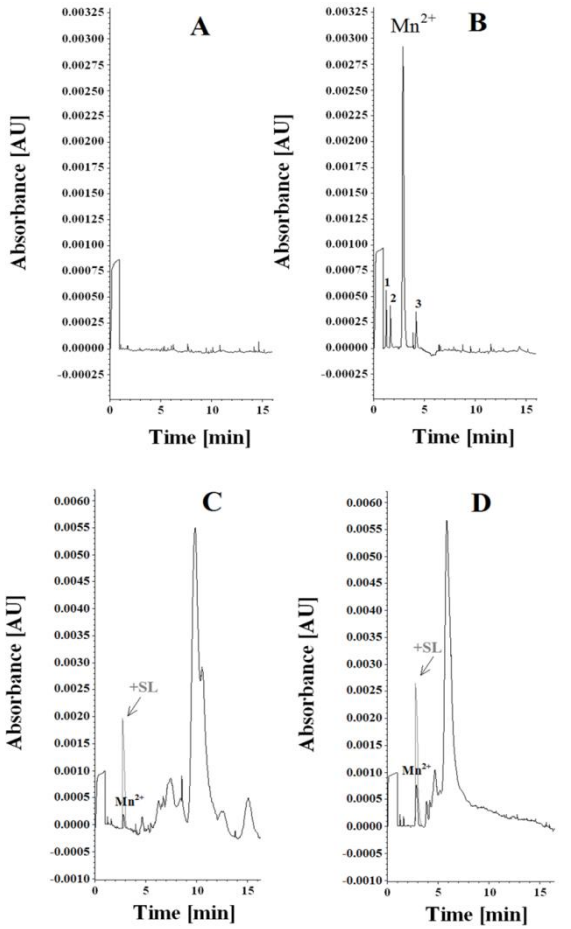

Figure 2. Specificity of the method. (A) clear water, (B) aqueous solution of Mn^{2+} at the concentration of 1000 µg/mL and 200 µg/mL of: Ca^{2+}(1), Mg^{2+}(2) and Cu^{2+}(3) (C) electropherogram of a sample from roots, +SL (standard solution of Mn^{2+} at the concentration of 500 µg/mL) (D) electropherogram of a sample from leaves, +SL (standard solution of Mn^{2+} at the concentration of 500 µg/mL).

Table 2. Recovery data of Mn^{2+} ions from standard solutions after purification procedure (n = 6).

Added amount	Observed amount	% Recovery	% RSD
µg/mL	µg/mL		
3	2.98±0.11	99.3	3.69
50	50.0±1.1	100.0	2.20
300	300.0±1.2	100.0	0.40

Based on the method used, it was shown that the content of Mn^{2+} in parsley leaves and roots were 237 µg/g and 54 µg/g, respectively (Table 3 and Figure 2).

Table 3. The content of manganese (II) ions in 1 g of leaves and roots.

Analyte	Content in leaves	% RSD	Content in roots	% RSD
	µg/g		µg/g	
Mn^{2+}	237±6	2.41	54.0±1.3	2.48

5. Discussion

In this work, a full optimization and validation of the method for the determination of manganese (II) ions in samples of plant origin was carried out. The tested parameters that influenced the quality of the obtained results were: analytical wavelength, buffer composition (its type and concentration), pH, voltage, and temperature. The choice of the analytical wavelength for manganese (II) ions was made on the basis of absorbance studies of two salts (chloride and sulphate) containing Mn^{2+} ions. In both cases, the best wavelength for the best detection of manganese(II) was 214 nm. The type of buffer and its concentration had a great influence on the determination of Mn^{2+} ions. Initially, a number of borate buffers of increasing concentration were used, however, these buffers caused the instability of the current intensity, which negatively affected the precise determination of the migration time of the determined

ions. The phosphate buffer turned out to be a much better system. However, also in this case, the effect of its concentration and pH played a large role in obtaining the best separation parameters. Both too low and too high concentrations of sodium diphosphate did not allow for sufficient separation to enable the detection of Mn^{2+} ions. A similar situation occurred when selecting the appropriate pH of the buffer, where the pH increase resulted in a significant extension of the analysis time and deterioration of the separation parameters. Another very important parameter was the voltage, the adjustment of which had a huge impact on the separation process. Low voltage values significantly extended the analysis time needed to detect manganese (II) ions. Voltage values exceeding 10 kV caused smaller or larger decreases in current intensity, which did not allow for a stable separation process. In this work, a very fast method of sample purification was also developed, thanks to which it was possible to get a very fast results of analysis.

6. Concluding remarks

The optimized CE method allows for the direct determination of manganese (II) in fresh parsley leaves and roots. Limit of detection and quantification for Mn^{2+}, for this method were 0.97 and 2.94 µg/mL, respectively. The developed CE method is a very fast, simple, and stable method that does not require the use of additional reagents to modify the buffer and separation process. This method may be an attractive alternative to other methods.

Author(s) contribution

Błażej Grodner is the originator and main contractor of the work. Błażej Grodner developed the method and conducted all the analyses. Małgorzata Jelińska performed the preparation and purification of analytical samples.

Conflict of interest

The authors declare no conflict of interest.

References

1. Pajarillo, E.; Nyarko-Danquah, I.; Adinew, G.; Rizor, A.; Michael Aschner, M.; and Lee, E. Chapter Six - Neurotoxicity mechanisms of manganese in the central nervous system. *Adv Neurotox*. **2021**, 5, 215-238.
2. Al-Fartusie, F.S.;, Mohssan, S.N. Essential Trace Elements and Their Vital Roles in Human Body. *Indian J Adv Chem Sci*. **2017**, 5(3), 127-136.
3. Bae, Y-J.; Kim, M-H. Manganese Supplementation Improves Mineral Density of the Spine and Femur and Serum Osteocalcin in Rats. *Biol Trace Elem Res*. **2008**, 124, 28–34.
4. Rondanelli, M.; Faliva, M.A.; Peroni, G.; Infantino, V.; Gasparri, C.; Iannello, G.; Perna, S.; Riva, A.; Petrangolini, G.; and Tartara, A. Essentiality of Manganese for Bone Health: An Overview and Update. *Nat Prod Commun*. **2021**, 16(5), 1-8.
5. Aschner, M.; Connor, J.R.; Dorman, D.C.; Malecki, E.A.; Vrana, K.E. Manganese in Health and Disease. In: Massaro, E.J. (eds) Handbook of Neurotoxicology; Humana Press, Totowa, NJ. **2002**, 195-209. https://doi.org/10.1007/978-1-59259-132-9_11
6. Soldin, O.P.; and Aschner, M. Effects of manganese on thyroid hormone homeostasis. *Neurotoxicology*. **2007**, 28(5), 951–956.
7. Grant, E.C.G. Epilepsy and manganese. *Lancet*. **2004**, 363, 572.
8. Bowman, A.B.; Kwakye, G.F.; Hernández, E.H.; Aschner, M. Role of manganese in neurodegenerative diseases. *J Trace Elem Med Biol*. **2011**, 25, 191–203.
9. Levy, B.S.; Nassetta, W.J. Neurologic Effects of Manganese in Humans. *Int J Occup Environ Health*. **2003**, 9(2), 153-163.
10. Cotzias, G.C.; Manganese in health and disease. *Physiol Rev*. **1958**, 38, 503–532.
11. Barbeau, A. Manganese and extrapyramidal disorders: a critical review and tribute to Dr. George C.Cotzias. *Neurotoxicology*. **1984**, 5, 13–36.
12. Huang, C.C.; Chu, N.S.; Lu, C.S. Chronic manganese intoxication. *Arch Neurol*. **1989**, 46, 1104–1106.
13. Hanf, L.; Henschel, J.; Diehl, M.; Winter, M.; Nowak, S. Mn^{2+} or Mn^{3+}? Investigating transition metal dissolution of manganese species in lithium ion battery electrolytes by capillary electrophoresis. *Electrophoresis*. **2020**, 41, 697–704.
14. Pomazal, K.; Prohaska, C.; Steffan, I.; Reich, G and Huber, J.F.K. Determination of Cu, Fe, Mn, and Zn in blood fractions by SEC-HPLC-ICP-AES coupling. *Analyst*, **1999**, 124, 657-663.
15. Hu, Q.; Yang, G.; Yang, J. and Yin, J. Study on determination of iron, cobalt, nickel, copper, zinc and manganese in drinking water by solid-phase extraction and RP-HPLC with 2-(2-quinolinylazo)-5-diethylaminophenol as precolumn derivatizing reagent. *J. Environ. Monit.*, **2002**, 4, 956–959.
16. Brewer, P.G.; Spencer, D.W. Colorimetric determination of manganese in anoxic waters. *Notes*. **1971**, 16, 107-110.
17. Chiswell, B.; Rauchle, G.; Pascoe, M. Spectrophotometric methods for the determination of manganese. *Talanta*. **1990**, 37(2), 237-259.
18. International Conference On Harmonisation Of Technical Requirements For Registration Of Pharmaceuticals For Human Use. ICH Harmonised Tripartite Guideline Validation Of Analytical Procedures: Text And Methodology Q2(R1) Current Step 4 version Parent Guideline dated 27 October 1994 (Complementary Guideline on Methodology dated 6 November 1996 incorporated in November 2005).
19. Guideline Bioanalytical method validation 21 July 2011 EMEA/CHMP/EWP/192217/2009 Rev. 1 Corr. 2** Committee for Medicinal Products for Human Use (CHMP). European Medicines Agency (2011).Brzeziński, T.; *Historia medycyny*, Wyd. II; Wydawnictwo Lekarskie PZWL, Warszawa, Polska, 1995; ISBN 83-200-184-3.