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CHARACTERIZATION OF PARAMAGNETIC IRON(III) AND MANGANESE(II) CONTENT OF SOME MEDICAL HERBS BY ELECTRON PARAMAGNETIC RESONANCE (EPR) SPECTROSCOPY

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Abstract:
Narrowleaf plantain (*Plantago lanceolata* L.), marigold (*Calendula officinalis* L.) and shepherd's purse (*Capsella bursa-pastoris* L. Medik) are really popular, useful herbs for several diseases because of their considerably high and diverse agent- and element content. The manganese and iron content were investigated in their paramagnetic form (Mn(II) and Fe(III)), as these oxidation states are very important in human metabolism. The paramagnetic forms of these ions were detected by Electron Paramagnetic Resonance (EPR) spectroscopy. For the quantitative analysis of Mn(II) the EPR signals were simulated by a computer program and the calculated double integral were compared with those of standard calibration series. In this way an effective quantitative method was implemented in order to determine the absolute Mn(II) content, which can be evolved later as a rutin technique. The Mn(II) content of the infusums were measured and these amounts were compared to the total Mn content determined previously by ICP-OES. It gives rise to the possible use as dietary intake for Mn(II). The Fe(III) content of the samples were investigated by frozen solution EPR measurements comparing these spectra with different Fe(III)complex standard solutions. The results pointed that Fe(III) may exist in Fe(III)citrate complex in the solutions. The concentrated, frosted infusums were prepared to reach a better sensitivity. From this kind of sample Cu(II) could also be detected beside Mn(II) and Fe(III). Fe(III) content was also determined in ground drug samples. Marigold was carried out to be the richest plant in Fe(III), and the next one was narrowleaf plantain.

Keywords: EPR spectroscopy, infusum, manganese(II), iron(III), narrowleaf plantain, marigold, shepherd's purse, tea

Introduction

Narrowleaf plantain (*Plantago lanceolata* L.), marigold (*Calendula officinalis* L.) and shepherd's purse (*Capsella bursa-pastoris* L. Medik) are really well-spread herbs all over the world for their beneficial effects on general inflammatory diseases. They have several active substances, and can be used for also internal and external treatments. In comparing with medicines, herbs are not examined in detailed, however they are used for the same target, and their biological activity can be very similar. In regard to this, investigation of the composition and mechanism of these plants is necessary (Kabata-Pendias & Mukherjee, 2007; Szentmihályi et al., 2008).

Metal ions strongly influence the inflammatory processes, that's why their quantitative and qualitative information in the extracts used in inflammatory diseases is suggested to be known. The important elements in these processes were measured for the three examined herbs by ICP-OES, and a part of it was reported in an other article (Rábai et al., 2012). They consist of a high amount of many essential elements, polyphenols, flavonoids and they all have high ferric reducing ability.

Manganese and iron are two especially dominant elements in human nutrition, which play a main role in several important processes in living organisms. They both have different oxidation forms, EPR-active forms among them. Manganese can be found in each cells. It is necessary for synthesis of lipids, carbohydrates, proteins and cholesterol, acts an important part in the metabolism of iron, and has significant antioxidant effect. Its usual oxidation states are II, III and IV, which can transform into each other. Mn(II) and Mn(III) can be absorbed by human body nevertheless the most important metabolic form of manganese is Mn(II) (Szentmihályi et al., 2006; Kopittke et al., 2013;Reaneay et al., 2002). Mn(II) is the main form in blood, and its absorption happens by active cotransport from foods (Donner et al., 2012). Manganese is in interaction with iron, their amount is influenced by each other. Iron is a component of many proteins, and essential for producting normocyte and haemoglobin. It has a significant role in function of immune and nervous system. Only iron(II) and (III) can be found in living organisms. Metabolism of hem-connected Fe(III) is more beneficial than metabolism of Fe(II) in human body, that is why iron utilization is better from red meat which is a richer source of hem-connected Fe(III) than other kinds of meat. A redox reaction with manganese controls the oxidation state of iron. Less manganese causes an increased amount of Fe(II) and manganese excess makes the iron oxidated into indissoluble Fe(III)-phosphate (Szentmihályi et al., 2004).

Mn(II) and Fe(III) are the most important forms in human metabolism, so the concentration of these and other forms in these samples can be a useful data taking into account the therapeutic usage of these plant extracts. EPR spectroscopy is able to detect species with unpaired electrons, so it is a perfect technique for detecting the paramagnetic form of these elements. It is usually used for structural research at physics, physico-chemistry, chemistry, geography and other fields of life. EPR is based on the separation of degenerated energy states of spins in magnetic field (electron-Zeeman-interaction).

This report is about measuring Mn(II) and Fe(III) content in extracts and ground drug of narrowleaf plantain, marigold and shepherd's purse. Due to previous experiments, EPR spectroscopy was often used for discovering stress tolerance (Labanowska et al., 2012; Bogushevich & Matveichuk, 2009), stability (Biyik & Tapramaz, 2009; Polat & Korkmaz,2007), oxidative stability (Thomsen et al., 2000; Vidovic et al., 2010; Yeretzian et al., 2013) or photosynthetic efficiency (Alberti et al., 2000; Yoshii et al., 1999) in living agricultural plants, as these paramagnetic substances

can be considered as an indicator of nutritional and weather conditions (Sanyal et al., 2012; Lisowski et al., 1993). However, only a few papers were about measuring Fe(III) and Mn(II) by EPR in plant samples (Bogushevich & Matveichuk, 2009, Polovka et al., 2003; Biyik &Tapramaz, 2010; Morsy & Khaled, 2001), but narrowleaf plantain, marigold and shepherd's purse have not been analyzed yet from the aspect of paramagnetic metal content. Our aim was to discover the paramagnetic element composition of these herbs to be able to determine their utilization in human consumption. We also aimed to develop a methodology for the measurement of these elements.

Materials and methods

Materials. Leaves of narrowleaf plantain *(Plantago lanceolata* L.) were produced by Herbária Patikája, trade from the producting series of 07533-07534, K-101/111. Flower of marigold (*Calendula officinalis* L.) was originated from Adamo fitt, Reflex Kft., 437812, 5/2010 and herba of shepherd's purse (*Capsella bursa-pastoris* L. Medik.) from the same trade, 16868090310, 4/2010.

Aqueous Extracts and Ground Samples. Freshly prepared *infusums* were used for measuring Mn(II). An amount of 1 g drug was taken into 20 mL hot bidistilled water, and was soaked for 5 min until the filtering. Alcoholic tinctures were also prepared with the same recipe with 10 % ethanol content, which were made to stand for 10 and 20 min. For Fe(III) measurement *frost-dried sample*s were made from 10 g drug taken and boiled for 5 min in 200 mL hot bidistilled water. The dried sample was dissolved in 4 mL bidistilled water and 0.5 mL methanol from which 100 μL sample and 25 μL methanol was filled into the capillary. *Ground* drugs were also examined for measuring Fe(III).

ICP-OES Measurement. Iron and maganese concentration of the samples was determined with an ICP-OES (inductively coupled plasma optical emission spectrometer). Type of instrument: Spectro Genesis ICP-OES (Kleve, Germany). After digestion of the samples (0.5 g from drug, 20 mL of evaporated extracts) with a mixture of nitric acid and hydrogen peroxide (10 + 4 mL) and dilution with deionised water to 20 mL (extracts) or 25 mL (drug), concentration of elements was determined (Szentmihályi & Then, 2000).

EPR Measurement. All EPR spectra were recorded with a BRUKER EleXsys E500 spectrometer (Figure 1). *Infusums* and Mn(II) standard solutions were measured in glass capillaries at 25 °C. 10-15 scans were taken from the sample using 100 kHz modulation frequency, 10 G modulation amplitud, and 10 mW microwave power. As Fe(III) content was expected to be very small (owing to the previously measured ICP-OES data[4]), concentrated, *frost-dried samples* were prepared for the EPR measurements. To increase the sensitivity of the method, the samples were frozen in liquid nitrogen and the measurements were performed at 77 K. These measurements were done in quartz capillary to avoid the disturbing Fe(III) signal of the glass. Fe(III) is detectable at around 1500 G with a half-field signal, which belongs to the $\Delta S=2$ forbidden transition.

Evaluation of the EPR Spectra. The baseline of the Mn(II) EPR spectra was first corrected by subtracting pure water signal recorded without manganese. Then the six-line spectra were simulated by the EPR computer program (Rockenbauer &Korecz, 1996), taking into account an electron spin quantum number $S_{eff}=1/2$, and nuclear spin quantum number $I=5/2$, with parameters of $g_0=1.999$, $A_0=95.5$G. For quantitative

analysis, the double integration were performed on these simulated spectra. This allowed that even at low concentration where the noise of the spectra was higher, a reliable integral data could be obtained. The Mn(II) concentrations of the *infusums* were calculated by the help of standard concentration series measuring the same way.

Fe(III) signals were analyzed by measuring the intensities of the signal belongs to the forbidden transition at half field (~ 1500 G). Concentrations were determined by the help of calibration standards.

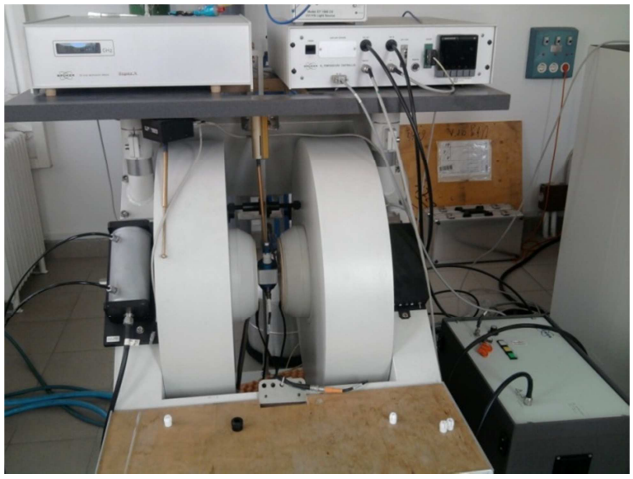

Figure 1. X-band CW EPR machine

Results and discussion

Mn(II) Determination. Mn(II) standard solutions were made from 0.2 to 1.5 µg/mL concentrations of Mn(II)-nitrate, and their EPR spectra were measured at room temperature, as it is shown in Figure 2. A calibration line could be fitted from the second integral of the EPR spectra which is related to the paramagnetic metal ion concentration. A good linearity is shown from 0.5 µg/mL to 1.5 µg/mL, the regression coefficient is 0.9913 as it can be seen in Figure 3.

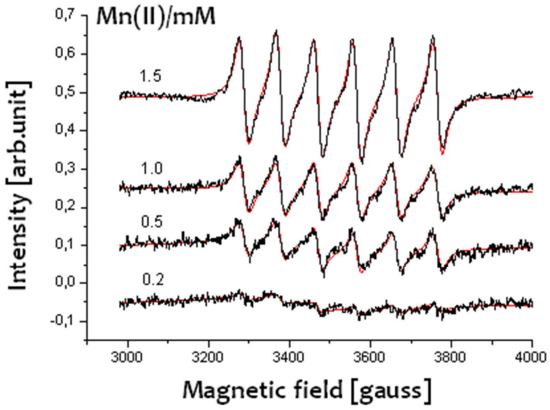

Figure 2. Spectra of Mn(II) standards.

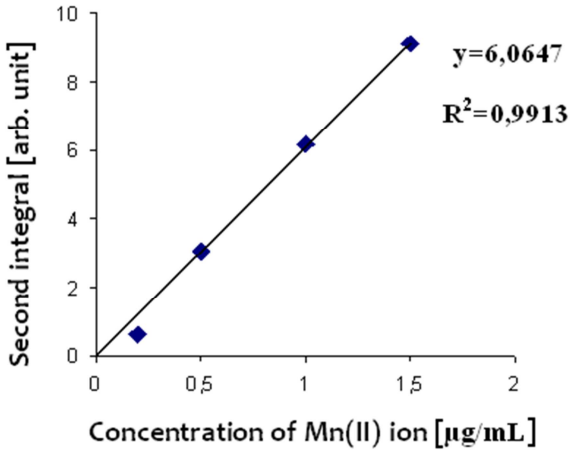

Figure 3. Calibration of Mn(II).

For the fresh-prepared aqueous *infusum* made of narrowleaf plantain the measured EPR spectrum is shown in Figure 4. The spectrum characteristic of the Mn(II) ion showed a manganese hyperfine splitting constant of 95.5 G, and possibly originate from Mn(II)-aqua complex. The concentration could be determined from the calibration curve, which was found to be 0.35 µg/mL in the *infusum*. The total manganese concentration was 0.658+/-0.26 µg/mL in narrowleaf plantain tea according to the ICP-OES measurement, which means that the rate of paramagnetic Mn(II) to the total manganese content is 53.19%. The rates for the other extracts are presented in Table 1. Using ethanol extracts instead of pure water the manganese(II) content increased significantly for narrowleaf plantain solution. In all the three ethanol extracts of herbs, we could measure higher Mn(II) content for solutions standing for 20 min instead of 10 min. The highest Mn(II) content of all was found for the extract of narrowleaf plantain with 10% ethanol standing for 20 min.

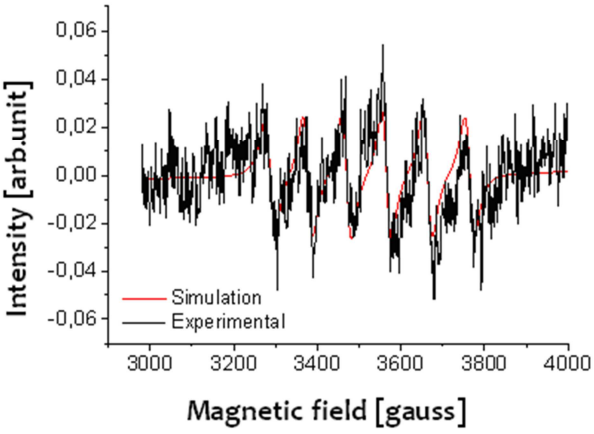

Figure 4. Mn(II) spectrum of the narrowleaf plantain infusum.

Table 1. Total and paramagnetic concentration of manganese in different extracts

Extracts	Total Mn content by ICP-OES (µg/mL)	EPR result (µg/mL)	Mn(II) content(%)
Narrowleaf plantain (aqueous tea)	0.658	0.35	53.19
Narrowleaf plantain 10 min 10% ethanol	0.809	0.496	61.31
Narrowleaf plantain 20 min 10% ethanol	0.733	0.567	77.35
Marigold for 10 min 10% ethanol	0.386	0.159	41.19
Marigold for 20 min 10% ethanol	0.413	0.166	40.19
Shepherd's purse for 10 min 10% ethanol	0.451	0.166	36.80
Shepherd's purse 20 min 10% ethanol	0.446	0.236	52.91

Fe(III) Determination. The *frost-dried sample*s were measured at 77 K, beside Fe(III) and Mn(II), Cu(II) signal were also detected. In the spectrum, a typical signal of an elongated octahedral Cu(II)complex can be discovered (Figure 5), and the Mn(II) lines are also noticable in frost-samples. The broad singlet background signal also reports the presence of Fe(III). Simulation of the *frost-dried sample* can be seen on Figure 6, the Fe(III)/Cu(II) ratio is 32 measured by EPR, while ICP OES data show a smaller ratio of 20.5.

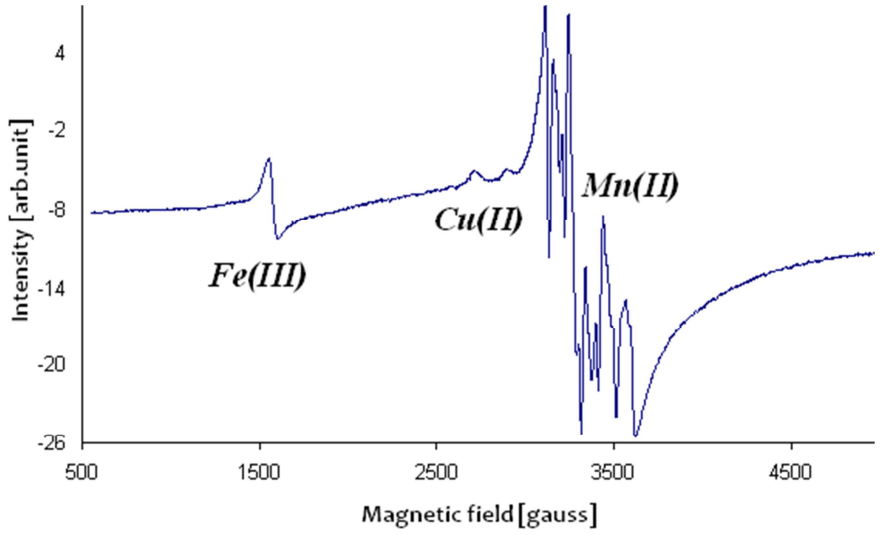

Figure 5. Spectrum of the frost-dried narrowleaf plantain infusum.

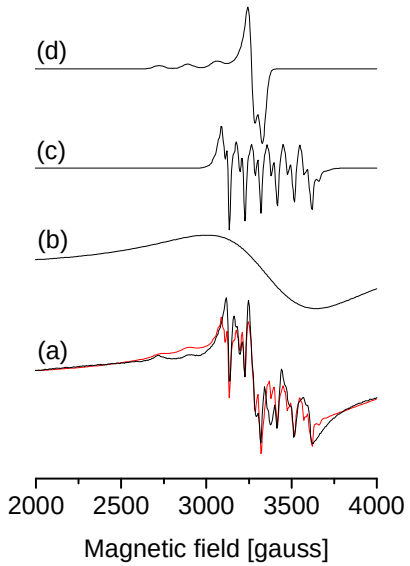

Figure 6. Simulation of the frost-dried narrowleaf plantain infusum (a) measured (black) and simulated (red) curves taking into account the component spectra of (b) Fe(III) with parameters g_o=1.97, w=560 G, n_{Fe}/n = 94%, (c) Mn(II) g_x = 1.998, g_y = 2.026, g_z = 1.990, A_x = 94.6 G, A_z = 92.0 G, A_z = 96.9 G, n_{Mn}/n = 3% and (d) Cu(II) $g_{\parallel}$ = 2.265, $g_{\perp}$ = 2.058, $A_{\parallel}$ = 164.5 G, $A_{\perp}$ =7.9 G, n_{Cu}/n = 3% .

In order to measure the concentration of Fe(III) in solution samples, a calibration method was investigated. As the EPR spectra of Fe(III) contained solutions recorded at room temperature resulted in a very broad singlet spectrum, this measurement was not suitable for the quantitative analysis. In frozen solution (77 K) however the half field signal (at 1575 G, g = 4.27) in the EPR spectra could be adequate for this purpose. For Fe(III) determination in the *frost-dried samples* different types of Fe(III) were solved in bidistilled water, namely Fe(III)nitrate, Fe(III)ammonium-sulphate, Fe(III)-citrate, Fe(III)acetylacetonate, Fe(III)ammonium-oxalate, Fe(III)chloride, Fe(III)sulphate, Fe(III)oxalate and Fe(III)oxide, in order to compare their line shapes (Figure 7). As the low concentration, and therefore high background signal of these spectra made the double integration impossible, qualitative data could only be determined by using the intensities of the half field signals. The similar line shape of the choosen standard allows to use the intensity values instead of the double integration to determine the concentration data. The line shape of the half field signal indicates the difference between the coordination sphere of the different Fe(III)complexes (Morsy & Khaled, 2002). The highest similarity was found with Fe(III)citrate (Figure 7), which suppose that for the speciation of Fe(III) a citrate-complex form can be suggested in narrowleaf plantain.

An Fe(III)citrate standard series in range of 5 and 500 µg/mL was freshly prepared and measured for calibration. As a conclusion we deduced, that the concentration determination of Fe(III)complexes in frozen solution is highly uncertain owing to possible aggregation processes of Fe(III)complexes upon freezing. Therefore the reproducibility of the standard signal of 100 µg/mL was only ±30%. For this quantitative data could not be obtained for Fe(III) with this method.

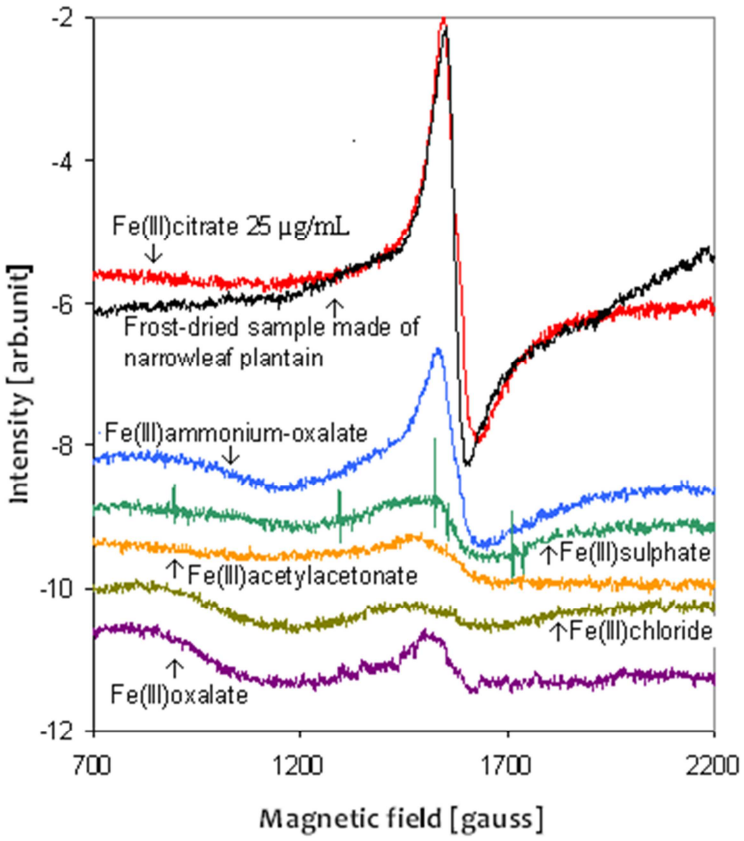

Figure 7. Spectra of Fe(III)-standard solutions.

Ground Samples. Three ground samples were also measured for detecting Fe(III). The reproducibility of measuring ground samples at room temperature was found to be similarly good as measuring the solutions at room temperature. However another problem occurs when absolute concentration should be measured, namely that Fe(III) is supposed to be in cluster form in drug, which means that spin-exchange coupling occurs between adjacent spins originating that the concentration is not correlated any more to the double integral of spectra. For this we doubt that the absolute concentration data of Fe(III) could be obtained with this method, however the relative concentration can be reported, and a priority can be carried out among these three herbs. The values of Fe(III) concentration can be seen on Table 2. The first column shows the values came from the intensity of the half field signal (1575 G, g = 4.27) referring to 1 g ground samples. The second column is shown just in aim for representing the total iron concentration, as ICP-OES determination is a better, quantitative analytical method. The ICP-OES information is originated from measurements of drug samples. Marigold sample has by far the highest Fe(III) concentration according to both kind of results (EPR and ICP-OES data). Total iron content of narrowleaf plantain is the second highest value, however its Fe(III) concentration is the lowest among these three herbs.

Table 2. Comparison of data earned by EPR and ICP-OES

Ground samples	Fe(III) intensity/g (calculated) [A. u.] by EPR	Total Fe concentration in drug [µg/g +/- SD] by ICP-OES
Marigold	2606	1721 ±480
Sheperd's purse	212	208 ±38
Narrowleaf plantain	190	280 ±43

The extracts and ground samples of narrowleaf plantain, marigold and shepherd's purse were first time measured by EPR in aim to discover their paramagnetic compounds, which are more beneficial to health than the other diamagnetic oxidation forms. Mn(II) EPR spectra were fitted by a computer program to earn precise, noiseless data for the calculation of double integral. For the quantitative analyzis of Mn(II) the EPR measurements of the solutions at room temperature can be suggested, instead of frozen solution, as the reproducibility is much higher. The methodology, developed to measure the concentration of Mn(II), can be suggested as a rutin technique for similar investigations. Marigold contains the less Mn(II), while narrowleaf plantain can be a really good source of it. In case of concentrated frost-dried samples beside Mn(II) and Fe(III), Cu(II) could also be detected by measuring the frozen solution at 77 K. EPR is suitable for identify Fe(III), but under our measuring conditions, we were unable to determine the concentration, because of bad reproducibility of the measurements at 77 K. Instead, ground samples of herbs were measured at room temperature and the relative concentration of three different ground herbs were compared. Marigold plant contains the most abundant Fe(III) among them. This work revealed possible methods to detect paramagnetic species in extracts and teas of herbs which can be rich sources of important micro elements in human consumption.

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