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Connection between measurement of vitamin B_{12} by (RP)HPLC-ICP-MS hyphenated analytical system and antioxidant capacity

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Vitamins constitute a diverse group of organic compounds essential in trace amounts for the proper growth and maintenance of life. They have different specific roles in metabolism, and their lack or excess can generate serious diseases. When it is necessaryTo ensure the adequate intake of vitamins the human diet can be completed with high range of multivitamin tablets and food products supplemented with vitamins, such as B_{12} fortified energy drinks. Each vitamin protects the human body from oxidizing agents, that's why it is of importance to determine the antioxidant capacity and amount of vitamin in food.

The aim of this study was to develop an (RP)HPLC-ICP-MS method for the determination of cyanocobalamin, the compound used for vitamin B_{12} fortification in food. Moreover we also measured the antioxidant capacity and total phenols in the energy drink sample.

In the first part of the work the reversed-phase HPLC separation was optimized with UV detection. After applying various chromatographic set-ups finally an Agilent Eclipse XDB 4.6 x 250mm (5um particle size) column having a C18 stationary phase was chosen. As mobile phases sodium-acetate (pH = 4.0) - acetonitrile and methanol - 0,05 V/V% trifluoracetic acid/ H_2O were used. We worked with two kinds of ICP-MS configuration, - with oxygen gas and without oxygen gas- and these methods were compared.

After the aqueous extraction and alcohol extraction of the energy drink the antioxidant capacity with FRAP assay and total phenol using reagent of Folin-Ciocalteu with spectrophotometer were determined.

The RP-HPLC-ICP-MS system was compared to HPLC-UV system. The selectivity of HPLC-ICP-MS is better, since the cyanocobalamin measurement based on its Co atom content, similarly the sensitivity of HPLC-ICP-MS is hundredfold better, according to the sensitivity of –UV detection. Moreover HPLC-ICP-MS without oxygen gas is more sensitive, then the other, but HPLC-ICP-MS with oxygen gas is occurred to be a more robust system.

The two extractions had a difference in efficiency, because the value of total phenol of alcohol extraction is twofold, as compared to the value of water extraction. The value of antioxidant capacity was too low according to total phenol, which means, that this assay can't measure the antioxidant effect of each phenol compound.

Both of cianocobalamin detection is good for measurement of B_{12} fortified food with simple matrix and vitamin tablet. But the selectivity of the UV detection wasn't enough in the case of energy drink, because the cianokobalamin coeluated with another compound, accordingly we couldn't determine amount of cianokobalamin properly. The ICP-MS detection is more sensitive, according to the UV detection and it measures the cyanocobalamin based on its Co atom content, so determination is more selective. These attributes allow to measure cianocobalamin in very small range, because amount of B_{12} in food is too low (ng/ml).

Phenolic composition and *in vitro* antioxidant activity correlation in *Sempervivum tectorum* extracts

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Sempervivum tectorum (Crassulaceae) is a widely known herb. In folk medicine its juice and leaves were used against inflammation of the ears. The juice was also applied to herpetic eruptions of the skin, minor burns and wounds. It showed remarkably potent antioxidant activity determined by chemiluminometric and EPR spin trapping methods, and inhibited lipid peroxidation induced both enzymatically and non-enzymatically.

Antioxidative, anti-inflammatory and antinociceptive effects of *Sempervivum* has been previously described, though the mode of action is still unexplained and any compounds has not been attributed to these effects. Phytochemical screening of *Sempervivum* extracts with different polarity proved the presence of notable quantity of polysaccharides, polyphenolic

compounds, flavonoids and organic acids. The purpose of this work is the comprehensive chemical analysis of these extracts, implying their fractionation, and the evaluation of their structure-activity relationship.

Extracts of lyophylized and powdered *Sempervivum* tectorum leaves with different polarity (water, 80% (v/v) methanol, methanol, ethanol, aceton, ethyl-acetate and chloroform) has been studied by LC-ESI-MS/MS. Towards a more detailed phytochemical analysis extracts prepared with methanol and chloroform have been fractionated by column chromatographic methods, and the fractions have been studied by LC-MS. *In vitro* antioxidant activity of extracts and fractions has been determined by spectrophotometry using DPPH (2,2-diphenyl-1-picrylhydrazyl) and ABTS (2,2'-azinobis-(3-ethyl-benzothiazolin-6-sulfonic acid) radical scavenging activity assays. Antioxidant activity has been characterized by $IC50^{-1}$ values.

The *Sempervivum* leaf extract, prepared by acetonic extraction and acidic hydrolysis had the highest antioxidant capacity. It contained purely flavonoid aglycones and its antioxidant activity was comparable to the one of kaempferol standard. The extract with the second highest antioxidant activity was prepared by 80% (v/v) methanol. Its LC-MS evaluation proved the presence of rutin, kaempferol-di(rhamno)-hexoside and five other kaempferol-glycoside derivatives. Houseleek extracts affected more potent radical scavenging activity against ABTS, than DPPH. Total polyphenolic content and antioxidant activity of *Sempervivum* extracts showed a significant correlation both ABTS and DPPH radicals (r^2=0,907 and r^2=0,967, respectively).

LC-MS evaluation of *Sempervivum* proved to have a high content of flavonoids and other phenolics, which are assumed to play an important role in the eminent scavenging activity of the extracts. On the basis of correlation between total polyphenol content and antioxidant activity of extracts it was concluded, that the remarkable scavenger activity of *Sempervivum* is due to the synergism of its polyphenolic compounds.

Reducing oxidative stress and leukocyte activation in reperfusion injury with controled reperfusion

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Reperfusion of the limbs after acute and persistent ischaemia is associated with high rates of morbidity and mortality despite complete revascularisation. Reconstruction of blood flow will induce reperfusion injury with oxidative stress and inflammatory responses. There are experimental evidences that modification of the initial reperfusion modalities can minimize this reperfusion injury.

In our study we aimed to confirm in an animal model that controled reperfusion (CR) can reduce oxidative srtess and leukocyte activation in reperfusion injury.

In our work we used 10 yorkshire pigs that were divided in two groups. All of the animals underwent a 4 hours infrarenal aortic occlusion: after anethesia we made median laparatomy and clamped the infrarenal abdominal aortae. In the first group after occlusion we removed the clamp, restored the blood flow and closed te wound. In the second group after ischaemia we made CR. CR consisted of 30-minute infusion of a crystalloid reperfusion solution that was mixed with oxygenated blood (the blood:reperfusion solution ratio was 1:1) distal to the occlusion. After this procedure we restored the normal blood reperfusion.

Blood samples were collected before occlusion, on the end of ischaemic period, and after reperfusion in the15th minute (from inferior caval vein), in the 1st and 24th hour, and on 7th day (from peripherial vein). To monitore the evoked oxidative stress superoxide-dismutase activity and reduced glutathion concentration were measured. The degree of lipidperoxidation was marked with the quantity of malondialdehyde. The inflammatory respons was marked with the measurement of leukocyte activation. PMA induced free radical production of the leukocytes was measured.

The lipidperoxidation was significantly lower in the early reperfusion in the CR group. CR also lead to a smaller depletion of the antioxidant enzymes. The speed and rate of free radical production of leukocytes were significantly lower in CR group ($p<0,05$).

The results from this study strongly suggest the hypothesis that the results of conventional embolectomy for acute, severe lower-limb ischemia can be improved by CR. The study was supported by OTKA- K67731.