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Determination of chromium, selenium, and molybdenum in a therapeutic diet

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Monitoring both the trace element and main element content of drugs forms part of their quality control. Chromium, selenium and molybdenum were determined in therapeutic diet samples by inductively coupled plasma/mass spectrometry (ICP-MS) and electrothermal atomic absorption spectrometry (ET-AAS). Samples were digested by high pressure microwave digestion or by ashing in oven. ICP-MS measurements have demonstrated that the chromium determination in liquid therapeutic diet should be estimated on the basis of ^{53}Cr. In solid samples in some cases the value for Cr was elevated in comparison with the Cr content found by ET-AAS. The content of selenium can be determined on the basis of ^{77}Se or ^{82}Se after appropriate interference correction. Molybdenum content was determined on the basis of ^{95}Mo. Control measurements were made by ET-AAS. For quality assurance purposes some of the samples were analyzed by a control laboratory.

1. Introduction

Therapeutic diets are used in the therapy of dyspepsia, food intolerance or metabolic disorders. Depending on their therapeutic effect the diets vary in composition and in their concentration of individual components. Most of them contain proteins or single aminoacids, hydrocarbons, fats, minerals and vitamins. Among the minerals additives, the main elements (Na, K, Cl, Ca, P, Mg) and the microelements (Fe, Zn, Cu, Mn, I, Cr, Mo, F, Se) can be found.

Chromium is an essential nutrient required for normal sugar and metabolism and acts primarily by accelerating the action of insulin. It is present in the entire body with the highest concentrations in the liver, kidney, spleen and bone. Although chromium is required only in very small amounts, our modern life has left many people short of chromium on a daily diet basis with many being hypoglycemic, pre-hypoglycemic or diabetic. Chromium is needed for energy, and to maintain stable blood sugar levels. Together with other substances, it controls both insulin as well as certain enzymes. It works with GTF (Glucose Tolerance Factor) when this hormone related agent enters the bloodstream because of an increase of insulin in the bloodstream. Because chromium is readily excreted in the urine, toxicity does not seem to be a problem. But, dermatitis has been noted, as well as gastrointestinal ulcers and also liver and kidney damage if taken in large dosages over prolonged periods.

Molybdenum assists in breaking down sulphite toxin build-ups in the body, and may prevent cavities. With these qualities, there may be evidence of properties of this nutrient. It assists the body by fighting nitrosamines, which are associated with cancer, and may help to prevent anaemia. It is needed for normal cell function and nitrogen metabolism.

Molybdenum deficiencies in older males have also been linked to impotence and may be valuable in fighting mouth and gum disorders. Molybdenum forms part of sulphite oxidase, an enzyme that breaks down sulphites. Deficiencies of molybdenum are identified by the absence of the three molybdenum enzymes. The deficiency of this element and the metabolic disorders are accompanied by abnormal excretion of sulphur metabolites, low uric acid concentrations, and elevated hypoxanthine and xanthine excretion. The absence of sulphite oxidase in metabolic disorder can lead to death at an early age. A dosage of up to 250 microgram per day is considered safe while 15 milligrams can border on toxicity. Dosages of more than 15 milligrams may be toxic and excess molybdenum in the body can interfere with the metabolism of in the body, can give symptoms of gout, and may cause diarrhoea, anaemia and slow growth.

One of the main activities of selenium is its anti-aging properties and its ability to help rid the body of free radicals, as well as toxic minerals such as mercury, lead and cadmium. It is helpful in fighting infections, promotes more energy in the body, and not only helps with alleviating menopausal symptoms in women, but also assists the male in producing healthy sperm.

Selenium is also used against arthritis and multiple sclerosis and if provided in adequate amounts it is thought to help prevent cancer as well. Tissue elasticity and pancreatic function are also dependent on this mineral.

As mentioned earlier, selenium is toxic and excessive quantities may result in hair loss, tooth decay, brittle nails,

Table 1: Determination range and limit for analytical procedures used

Isotope	Method	Range (ngl^{-1})	DL[a] (ngl^{-1})
^{53}Cr			5.0
^{52}Cr	ICP-MS	500–10 000	48.3
Cr	GF-AAS	800–8 000	5.1
^{77}Se		500–10 000	18.9
^{82}Se			18,9
^{95}Mo	ICP-MS		43.2

[a]determined based on 3 s of the blank measurements (n = 6)

white spots, poor appetite, sour taste in the mouth, loss of feeling in the hands and feet. Change in skin pigmentation and a garlic smell of the breath (WHO 1996).

Due to the range in which the microelements can be found in therapeutic diets only a few analytical techniques can be used, such as atomic absorption spectrometry (AAS) (Lendinez et al. 2001; Camara et al. 2000; Quinaia and Nobrega 1999), inductively coupled plasma/mass spectrometry (ICP-MS) (Vanhaecke et al. 2000; Łozak et al. 2002; Sołtyk and Fijałek 2000), inductively coupled plasma/emission spectrometry (ICP-AES) (Daskalova and Boevski 1999) and electroanalytical methods (Łozak and Fijałek 1998; Fijałek et al. 1998; Korolczuk 2000).

The aim of the present was to determine chromium, selenium and molybdenum in therapeutic diet in form of solutions and in water soluble powders by ICP-MS and GF-AAS after microwave digestion or oven ashing.

2. Investigations and results

Table 1 presents the detection limits for the determination of chromium, selenium and molybdenum by ICP-MS and ET-AAS. The relative standard deviation for the contents of the elements as determined by ICP-MS varied between 0.2 and 15%.

Fig. 1 shows the effect of the digestion method and the isotope determined on Cr and Mo contents obtained in liquid (Reconvan and Survimed) and solid (Humana SL and Humana MCT) therapeutic diets. The Cr content recommended by producers is about 2 times lower in liquid (6,7 and 5 μg/100 g) than in solid (15 and 20 μg/100 g) diets. Experimental values found for Cr by ICP-MS measurements after microwave digestion and based on isotope ^{52}Cr are in all cases significantly higher than the expected values. The difference between observed and expected values was highest for Humana SL, which is a product based on milk and soya. After correction of the data for ArC^+ interference more realistic data were obtained. No significant differences between found and expected values were observed for Cr determination by ICP-MS in samples after

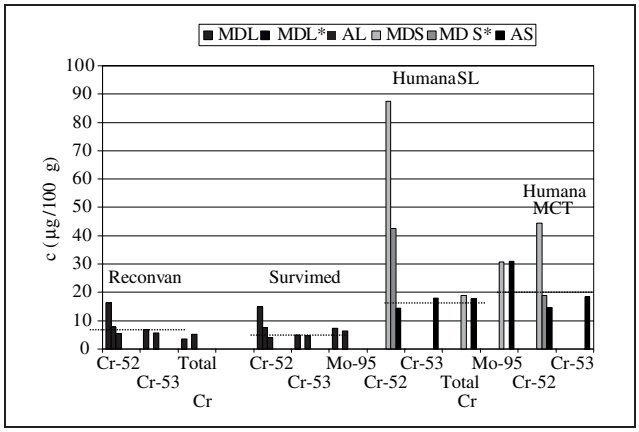

Fig. 1: Influence of the digestion method for liquid and solid samples on Cr and Mo contents

ashing in the oven. If the determination is based on ^{53}Cr, a difference between found and expected value was observed only in the case of Humana SL. The total Cr content determined by ET-AAS (Table 2) was close to the recommended value in all cases, independent of digestion method.

Fig. 2 presents a comparison between data obtained by ICP-MS measurements in two different laboratories and by ET-AAS. In the control laboratory (ICP-MS*) pressure digestion in PTFE vessels with conventional heating was used. The digestion temperature was 150 °C. No significant differences between ICP-MS measurements of Mo and Se content and other techniques were observed. For Cr the ICP-MS measurements were significantly higher than the values obtained by ET-AAS.

Natural chromium is a mixture of four isotopes: ^{50}Cr, ^{52}Cr, ^{53}Cr, and ^{54}Cr with relative frequencies of 4.3%, 83.8%, 9.5% and 2.4%, respectively. In ICP-MS measurements the signals from each isotope are disturbed. At the beginning of our study two Cr isotopes were used for the determination: ^{52}Cr and ^{53}Cr. Unfortunately carbon isotopes ^{12}C and ^{13}C make an ion ArC^+ with ^{40}Ar. This ion with a mass of 52 or 53 interferes with isotopes ^{52}Cr and ^{53}Cr, resulting in an increase in the amounts of chromium detected. This interference is mostly concentrated on the isotope ^{52}Cr due to the fact that 98,89% of natural carbon is present in the form of ^{12}C (Violante et al. 1998; Krushevska et al. 1998).

The chromium content found in samples of Humana SL determined on the basis of isotope ^{52}Cr is two times higher than the concentration detected on the basis of isotope ^{53}Cr. If a correction for the interference was introduced into the calculation of chromium content similar values were obtained for both chromium isotopes.

Selenium has six isotopes (^{74}Se, ^{76}Se, ^{77}Se, ^{78}Se, ^{80}Se and ^{82}Se). Analytical signals of all of them were interfered with by the matrix or by argon plasma. For Se determination in

Table 2: Elemental determination in CRMs

Isotop	Green algae								Oriental tobacco leaves	
	ICP-MS (mg/kg)				GF-AAS (mg/kg)				ICP-MS (mg/kg)	
	MW	RSD	Ashing	RSD	MW	RSD	Ashing	RSD		RSD
^{53}Cr	1.97 ± 0.04	2.0	1.77 ± 0.01	0.1	1.98 ± 0.03	1.4	2.15 ± 0.13	5.2	2.58 ± 0.06	1.6
^{52}Cr	2.48 ± 0.10	3.4	1.71 ± 0.01	0.2						
^{52}Cr	2.14 ± 0.08	3.6	–	–						
$^{77,82}Se$	–	–	–	–	–	–	–	–	0.14 ± 0,01	4.9
^{95}Mo	2.60 ± 0.08	2.8	–	–	–	–	–	–		

(P-ACHK No 12-2-02, certified values: [Cr] = 2.37 ± 0.42 μg/g; [Mo] = 2.28 ± 0.55 μg/g) and Oriental tobacco leaves (CTA-OTL-1, certified values: [Cr] = 2.59 ± 0.32 μg/g; [Se] = 0.153 ± 0.018) (n = 6)

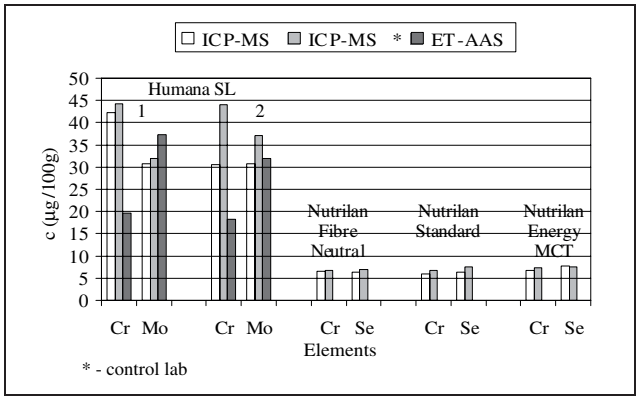

Fig. 2: Elemental concentrations found by ICP-MS and ET-AAS in liquid and solid samples

therapeutic diets the isotopes ^{77}Se and ^{82}Se with the natural fraction of 7.5% and 8.0% were selected. The most important interference with ^{77}Se is the interference from $^{40}Ar^{37}Cl$ which increases the determined Se content by about 6.4%. For ^{82}Se, interference from ^{82}Kr can change the determined Se content by less than 2%. Data presented in Fig. 2 demonstrate the good agreement between Se data corrected for interferences and data found in the control laboratory.

In nature Mo has seven isotopes (^{92}Mo, ^{94}Mo, ^{95}Mo, ^{96}Mo, ^{97}Mo, ^{98}Mo and ^{100}Mo). The Mo content in the analysed samples is based on the determination of ^{95}Mo (natural fraction: 14.7%). The analytical signal of this isotope is free from interferences. From the data obtained (Fig. 2) it is obvious that sample preparation (digestion) has no influence on the Mo content determined. Also good agreement is observed between our results and the values found by the control laboratory.

Due to the lack of appropriate therapeutic drug reference materials for validation of the method two other certified reference materials were used: Green Algae (P-ACHK No. 12-2-02) and Oriental Tobacco Leaves (CTA-OTL-1). In Table 2 the values obtained for Cr, Se and Mo determination by the procedure described are presented. No significant differences were observed between certified and found values for any of the elements.

3. Discussion

The residual carbon in the digestion solution after microwave digestion influences the Cr determination by ICP-MS based on ^{52}Cr due to the interference by the ion of mass 52, ArC^+. For this reason it is preferable to use the isotope ^{53}Cr for chromium determination. For Se and especially for Mo, no interferences with the analytical results due to digestion method were observed.

Ashing at 550 °C removed all the carbon from the sample but the loss of some chromium is possible. The ashing procedure is time consuming and significantly increases the determination time but the elemental contents found need no corrections.

ICP-MS can be used for simultaneous determination of several elements in one step using a standard addition method. In this case the variation coefficient is less than 15%.

4. Experimental

4.1. Equipment

The ICP-MS determination was performed with a VG PlasmaQuad 3 (Thermo Elemental, Franklin, MA, USA) and ET-AAS measurements with a Solaar M6 (Thermo Elemental, Franklin, MA, USA) using whole cathode lamp Cr-HCL and extended lifetime cuvettes. The sample digestion was done by a UniClever BM microwave digestor (Plazmatronika, Wrocław, Poland) or by a Mas 7000 microwave oven (CEM, Kamp-Lintfort, Germany).

Table 3: Parameters for microwave digestion

Step	Heating time (s)	Cooling time (s)	Power (W)
1	120	120	50
2	120	600	90
3	120	120	120
4	120	600	160

4.2. Samples

The following solid therapeutic diets were analysed: sample I: Humana SL (batch 13032002); sample II: Humana SL (batch 26042002) and Humana MCT (Humana Milchwerke Westfalen, Germany).

The following liquid therapeutic diets were analysed: Nutrilan Fibre Neutral, Nutrilan Standard Flavour Vanilla, Nutrilan Energy MCT Flavour Vanilla (Nutrichem Diät + Pharma GmbH, Roth, Germany); Survimed and Reconvan (Fresenius Kabi, Bad Homburg, Germany)

4.3. Chemicals

Standard solutions of chromium, selenium and molybdenum were prepared from stock solutions of 1 mg ml^{-1} (Merck, Darmstadt, Germany). Digestion was done using nitric acid (for ICP-MS, Merck). Other chemicals: $Mg(NO_3)_2$ 6 H_2O (Suprapur, Merck); deionized water (Nanopure Deionization System, Barnstead, Dubuque, Iowa, USA); Ar (99,999% vol. Praxair, Ƙdzierzyn, Poland).

4.4. Sample preparation

Microwave digestion: about 1–8 g of sample were weighed into one PTFE beaker (110 ml) and the same amount of sample with the addition of the appropriate standard solution was weighed into the second beaker. After addition of 3 or 5 ml of nitric acid to each beaker the mixture was stored at room temperature for 24 h. Microwave digestion was done in a closed system using a 4 step power input, with a maximum of 160 W (Table 3). Under these conditions the maximum pressure was 45 bar and the temperature was no higher than 155 °C. The digestion solution was transfered into a 100 ml volumetric flask As an internal standard 100 µ of 10 mgl^{-1} cobalt solution was added to each volumetric flask and made up with water.

Ashing in the oven: about 1–8 g of sample was weighed into the first quartz vessel and to the second and third vessels the same amount of sample, with the addition of an appropriate volume of standard solution were weighed and 5 ml 0,1% magnesium nitrate were added to each vessel. The mixture in the vessels was homogenized by an ultrasonic bath. The solution was then evaporated to dryness over a water bath at 100 °C. Ashing was made at 550 °C in a microwave oven over 8 h. The ash was dissolved in a 0.1% nitric acid and transferred into a 100 ml volumetric flask, 100 µl of 10 mg l^{-1} cobalt solution were added as an internal standard and the volumetric flask was made up with 0,1% nitric acid.

4.5. Elemental determinations by ICP-MS

Determination parameters: excitation power of plasma 1380 W, flow rate for gases: plasma 2.6–13.0 l·min^{-1}, nebulizer 0.74–0.80 l·min^{-1} and auxiliary 0.7–0.8 l·min^{-1}; base line of the background below 10 cps, amount of doubly charged ions 70/140 Ce^{2+}/Ce and 69/138 Ba^{2+}/Ba below 3.0%; amount of oxide ions 156/140 CeO/Ce below 3% and that of 154/138 BaO/Ba below 0.2%; aspiration time of a sample 180 s, measurement time 15 s in threefold repetition. Cobalt at a concentration of 10 µgl^{-1} was used as an internal standard in the determinations.

4.6. Cr determination by GF-AAS

The determination of Cr was performed in a pyrolytically coated graphite tube. The background Smith-Hieftje correction was applied at a current intensity of the lamp of 12 mA. The measurements were performed at a wavelength of 357.9 nm, the slit width being 0.5 nm (Table 4).

Table 4: Parameters for Cr determination by ET-AAS

Step	Description	Temp. (°C)	Time (s)	Flow (l min^{-1})
1	drying	100	20	0.2
2	ashing	1300	20	0.2
3	atomisation	2500	3	0
4	cleaning	2700	3	0.2

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