Biological Trace Element Research (2019) 191:517–521
https://doi.org/10.1007/s12011-019-1643-8

Safety Assessment of the Trace Element Impurities Ni and Cr in Pharmaceutical Herbal Products for Teething from Polish Pharmacies

Kamil Jurowski[1] · Mirosław Krośniak[2] · Maria Fołta[2] · Barbara Tatar[2] · Michael Cole[3] · Wojciech Piekoszewski[4,5]

Received: 13 December 2018 / Accepted: 16 January 2019 / Published online: 25 January 2019

Abstract

Control of elemental impurities (EIs) in pharmaceutical herbal products is currently important but not a very popular topic in modern toxicological analysis. The occurrence and concentration of EIs in the pharmaceutical herbal products should be controlled and meet the standards of directive International Conference on Harmonisation's Q3D Guideline on Elemental Impurities. An interesting area of interest is measuring EIs including toxic and allergic metals in pharmaceutical herbal products for teething. The aim of this article was determination of Ni and Cr impurities in pharmaceutical herbal products for teething available in Polish pharmacies. Justifications were (1) herbs as an important source of EIs and (2) infants may be particularly sensitive to the toxic effects of metals because they tend to absorb a higher fraction of an oral dose. The analysis was carried out using microwave-assisted wet digestion with concentrated nitric acid and electrothermal atomisation atomic absorption spectrometry. The safety assessment involved a triple approach: (1) level of Ni and Cr impurities in pharmaceutical samples; (2) level of Ni and Cr impurities including one-time administration of teething gels and (3) daily intake of metals. In all three cases, the results indicate that the standards of directive ICH Q3D are met for Ni and Cr. Overall, it can be concluded that none of the teething gels represents a health hazard to infants.

Keywords Elemental impurities · Safety assessment · Nickel · Chromium · Teething gels · Infants

Abbreviations

AAS	Atomic absorption spectrometry technique
CRM	Certified reference material
ET AAS	Electrothermal atomisation atomic absorption spectrometry
EIs	Elemental impurities
IARC	International Agency for Research on Cancer;
ICH Q3D	International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use
PDE	Permitted daily exposure
SD	Standard deviation
US EPA	The United States Environmental Protection Agency
WHO	World Health Organisation

Independent Researcher, Poland

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s12011-019-1643-8) contains supplementary material, which is available to authorized users.

✉ Kamil Jurowski
kamil.jurowski@gmail.com

[1] Faculty of Health Promotion, Kraków Higher School of Health Promotion, Krowoderska 73, 31-158 Kraków, Poland

[2] Department of Food Chemistry and Nutrition, Medical College, Jagiellonian University, Medyczna 9, 30-688 Kraków, Poland

[3] Faculty of Science and Technology, Anglia Ruskin University, East Road, Cambridge, UK

[4] Department of Analytical Chemistry, Faculty of Chemistry, Jagiellonian University in Kraków, Gronostajowa 2, 30–387 Kraków, Poland

[5] School of Biomedicine, Far Eastern Federal University, Sukhanova 8, Vladivostok 690950, Russia

Introduction

The elemental impurities (EIs) in pharmaceutical products must be considered in a safety assessment of the product appropriate for their intended route of administration. This problem is important, but there is a dearth of information around these products in the public domain literature. There are a number of potential sources of elemental impurities including herb and plant materials used in the manufacturing process, residual catalysts, metal reagent residues, or impurities through interactions with processing equipment. Since EIs do not provide any therapeutic benefit to the patient, their

levels in the pharmaceuticals should be controlled and monitored [1]. Moreover, EIs must be identified and determined in comparison to maximum acceptable levels to which a patient should be exposed. Given this, studies around monitoring and control of these impurities in pharmaceuticals are very important and present a challenge in modern toxicological analysis.

Whilst EIs from residual catalysts, metal reagent residues or impurities through interactions with processing equipment are monitored, EIs from plant ingredients are not a frequent subject of analysis. An interesting example of pharmaceuticals in which the real and important source of EIs is that of the plant materials used in teething remedies for infants—especially pharmaceutical herbal products [2]. Undoubtedly, the teething gels are the most popular teething remedies available in pharmacies [3]. Among the many metals, constituting impurities in teething gels, especially nickel (Ni) and chromium (Cr) presents an intriguing challenge.

Nickel is nutritionally not essential for humans [1]. Additionally, humans generally become sensitised to this element after prolonged contact with the skin [4]. Moreover, an oral challenge to a single dose of Ni administered in drinking water can induce dermatitis in nickel-sensitised individuals [4].

On the other hand, Cr is a very problematic element due to the number of its oxidation states, especially Cr(III) and also Cr(VI). It should be emphasised that currently, this element can only be considered pharmacologically active and not an essential element [5]. Cr(III) is the most abundant form in the environment, and is an essential element that plays a role in glucose metabolism. Cr(III) deficiency causes changes in the metabolism of glucose and lipids and may be associated with maturity-onset diabetes, cardiovascular diseases, and nervous system disorders [6]. On the other hand, soluble Cr(III) compounds are moderately toxic, rodent LD_{50} values being 100–400 mg/kg bw. In turn, Cr(VI) compounds are man-made and do not occur naturally in the environment. It should be noted that Cr(VI) has been shown to be genotoxic (animal studies via inhalation), and human epidemiological studies have shown Cr(VI) to be carcinogenic in the respiratory tract [6, 7]. However, there is no clear evidence of carcinogenicity where chromium has been tested in rats via the oral route. Moreover, there are some evidences that Cr(VI) is reduced to Cr(III) in the gastrointestinal tract, and so, only intakes that exceed the reducing capacity of the stomach will result in significant absorption of Cr(VI) across the gastrointestinal mucosa [7]. On the other hand, a portion of Cr(VI) evades the reductive detoxification and reaches target tissues [8]. It should be noted that any amount of Cr(VI) entering cells has the potential to initiate tumour formation [8]. Hence, Cr(VI) levels in drinking water must be set at levels that protect the entire population (it is important especially for infants) and they should be based on the Cr(VI) and not total Cr [8]. However, in most cases, Cr(III) and Cr(VI) residues in pharmaceuticals are usually measured as total Cr level. On the other hand, Directive 2006/141/EC, on infant and follow-on formulae, does not set minimum and maximum levels for this element [9].

The aim of this article was determination of Ni and Cr as EIs at trace level in pharmaceutical herbal products for teething available in pharmacies in Poland. This article is a continuation of our previous studies about determination of heavy metals (Pb and Cd) in this same samples [10]. This study included five of the most available and popular teething gels in Poland based on consultations with pharmacists and paediatricians. The Ni and Cr content of samples were determined by atomic absorption spectrometry using electrothermal atomisation (ET AAS) after microwave-assisted wet digestion with concentrated nitric acid. To the best of our knowledge, the Ni and Cr levels in pharmaceutical herbal products for teething are reported here for the first time.

Materials and Methods

Chemicals and Reagents

All solutions were prepared with ultrapure demineralised water that had been obtained by Milli-Q water purification system (Millipore, Bedford, MA, USA). Concentrated (65%) nitric acid for microwave digestion was of spectroscopic grade from Merck (Darmstadt, Germany). The purge gas was argon at purity 99.99%. The certified reference material (Corn Flour, INCT-CF-3) was purchased from the Institute of Nuclear Chemistry and Technology—Department of Analytical Chemistry (Warsaw, Poland).

Working solutions of Ni and Cr were prepared from the stock solutions of 1000 μg/mL (1 mg/mL) using ultrapure demineralised water in 0.5 mol/L nitric acid: nickel(II) nitrate and chromium(III) nitrate. Five working solutions with a concentration of 0.0, 12.5, 20.0, 50.0 and 100.0 μg/L were prepared and used for the analytical calibration.

Sample Collection and Pretreatment

The five teething gel containing herbs used in this study were purchased from Polish pharmacies in Malopolska region (Kraków and Niepołomice). The justifications of choice were availability and popularity of these products. All investigated pharmaceuticals are currently recommended by paediatricians. At the beginning of sample preparation, all products were coded (as A, B, C, D and E) to maintain appropriate sample tracking. The detailed information about the declared ingredients in the analysed teething gels tested in this study are described in Supplementary Material 1.

Before analysis, each teething gel was homogenised. Because all of the teething gels had an aluminium lid which could be a potential source of EIs, the first few centimetres of

each gel from the tube was discarded. Of each teething gel, 0.3 g was measured, poured into Teflon vessels and digested with 5.0 mL of concentrated nitric acid (HNO_3, 63%). The closed vessels were microwaved after 2 h. The samples were digested using microwave digestion system (CEM, Matthews, NC, USA). The detailed information about digestion procedure are described briefly in Supplementary Material 2. The samples were later cooled at room temperature (25 °C), and the final volume was made to 20 mL using ultrapure demineralised water. The cooled samples were stored in plastic bottles as stock sample solutions until analysis. Five replications were kept and done for all samples to increase the precision of the result.

Sample Analysis

The determination of Ni and Cr in the pharmaceutical samples was carried out using a Perkin-Elmer 5100 ZL atomic absorption spectrometer (Perkin-Elmer, Norwalk, CT, USA) with Zeeman background correction and with electrothermal atomisation (ET AAS technique). The time-temperature programme in the graphite furnace atomic absorption spectrometer for Ni and Cr determination is detailed as described in Supplementary Material 3. The emission source was hollow-cathode lamps for nickel (228.8 nm, 5 mA) and chromium (357.9 nm, 8 mA). Analytical quality control was performed using certified reference material (Corn Flour, INCT-CF-3): for Ni: 0.383 mg/kg certified value, and 0.386 mg/kg measured value; for Cr: 0.137 mg/kg certified value and 0.134 mg/kg measured value. The recoveries were 100.8% and 98% for Ni and Cr, respectively. The recoveries were calculated as the quotient of the determined level and the known amount of the determined element expressed as a percentage. The LODs for the metals were 1.93 μg/L for Ni and 1.65 μg/L for Cr. The LOQs for the metals were 5.79 μg/L for Ni and 4.95 μg/L for Cr. Calibration functions for all metals indicated good correlation coefficients (R) greater than 0.998 ($R_{Ni} = 0.9985$; $R_{Cr} = 0.9991$). This implies that there was good linearity of instrumental response with metal concentrations. Moreover, the quality control and validation of applied methodology are confirmed by previously described studies using the same methodology and apparatus [10, 11]. Five replications were performed for each sample. Data were analysed using Educational Analysis Set SAS® 9 licenced by the Jagiellonian University in Krakow. The mean and RSD were calculated.

Results and Discussion

Level of Ni and Cr Impurities in Pharmaceutical Samples

The concentrations of Ni and Cr impurities in all samples are shown in Table 1 as an average and %RSD.

Table 1 The levels of Ni and Cr in the analysed teething gels (μg kg^{-1})

Sample		Level, μg/kg			
		Ni		Cr	
No.	Code	Mean	SD	Mean	SD
1.	A	72.54	8.85	8.58	0.95
2.	B	82.22	5.85	8.74	0.73
3.	C	24.04	2.21	4.95	0.82
4.	D	116.98	6.33	4.42	0.54
5.	E	48.89	5.87	8.30	0.77

SD standard deviation

Ni and Cr were present in all of the products. It should be noted that Ni levels were approximately ten times higher (mean = 68.01 μg/kg) than Cr levels (mean = 6.99 μg/kg). The highest level of Ni was in sample D (115.02 ± 3.22 μg/kg) and the lowest level was in sample C (25.09 ± 2.25 μg/kg).

It should be noted that Ni is genotoxic but not mutagenic. When considering all forms of Ni, the IARC (International Agency for Research on Cancer) classified Ni as a human carcinogen (Group 1) [12]. Ingestion of large levels of Ni may cause stomach pain, depression of body weight and adverse effects on blood and kidneys [1]. Humans generally become sensitised to this metal after prolonged contact with the skin. A single oral challenge of Ni administered in drinking water can induce dermatitis in Ni-sensitised individuals [4]. Based on permitted concentrations for Ni impurities in pharmaceutical products (oral concentration) recommended by directive ICH Q3D (20 μg/g (20 mg/kg [1]), all of the samples analysed in this study meet the guidelines.

On the other hand, sources of Cr impurities in pharmaceuticals may include colourants, leaching from equipment or container closure systems, and catalysts [1]. Except when it is used as a catalyst, intake of Cr from pharmaceuticals will be in the form of metallic Cr-Cr(0) or Cr(III) rather than the more toxic Cr(VI); therefore, for drug products, the safety assessment is based on the known toxicity of Cr(III) and Cr(VI). However, since AAS does not take into account the phenomenon of

Table 2 The level of Ni and Cr to which the patient is exposed for one-time administration of the teething gels (ng/0.15 g)

Sample		Concentration, ng/0.15 g	
No.	Code	Ni Mean	Cr Mean
1.	A	10.88	1.29
2.	B	12.33	1.310
3.	C	3.61	0.7
4.	D	17.55	0.66
5.	E	7.33	1.24

Table 3 The daily exposure of Ni and Cr from teething gels (ng/day)

Sample		Daily exposure, ng/day	
No.	Code	Ni Mean	Cr Mean
1.	A	65.29	7.72
2.	B	73.99	7.86
3.	C	21.64	4.46
4.	D	105.28	3.98
5.	E	44.00	7.47

speciation, consideration of levels of each Cr form is not possible. Hence, considering acceptable limits for impurities including all Cr forms in pharmaceuticals via the oral route, recommended by directive ICH Q3D (1100 μg/g (1100 mg/kg [1]), all of teething gels (see Table 1) meet the guidelines.

Level of Ni and Cr Impurities Including One-Time Administration of Teething Gels

Level of Ni and Cr impurities including one-time administration of teething gels are needed for the assessment of these metals' exposure in one-time administration of applied pharmaceuticals. Based on the manufacturer's information, the teething gels as a pea-sized drop should be applied to the painful area with a clean finger. The average mass of one drop was measured as approximately 0.15 g of each gel. The obtained results of Ni and Cr impurities considering the one-time administration of teething gels are shown in Table 2.

Daily Intake of Metals

Considering the information in the leaflet for each product, the frequency of application should be no more than six times per day—especially after meals and before falling asleep. The daily exposure of Ni and Cr from teething gels is shown in Table 3.

The daily exposure of nickel is variable (22.58–103.52 ng/day). In turn, the daily exposure of chromium is relatively constant between gels (3.99–7.94 ng/day).

Based on a report published by the US EPA (the United States Environmental Protection Agency), the daily intake of nickel ranges from 100 to 300 μg/day [13]. However, the oral permitted daily exposure (PDE) for this element is 220 μg/day. All analysed samples in this study result in a dose below the PDE (Table 3). Additionally, considering the PDE for chromium based on ICH Q3D [1] i.e. 10,700 μg/day, all of the results are below the PDE (see Table 3).

Conclusions and Recommendations

The levels of investigated MIs in all of the teething gels analysed in this study are very low. The level of analysed metals considering one-time administration of teething gels is also very low, hence, is not a threat for infants. The daily exposure of Ni and Cr meets the standards of directive ICH Q3D. It can be concluded that none of the investigated gels in our study represents a health hazard for infants. Pharmaceutical herbal products for teething from Polish pharmacies are safe for infants.

However, a broader study considering other EIs (especially As, Hg) in pharmaceutical herbal products available in Polish market and pharmacies will be valuable as our previously published about chosen heavy metals (Pb and Cd) [10] or wider studies about toxic metals in commonly used pharmaceutical herbal products for infants like studies recently described by Alhusban et al. [14].

Compliance with Ethical Standards

This article does not contain any studies with human participants or animals performed by any of the authors.

Conflict of Interest The authors declare that they have no conflict of interest

References

1. US Food and Drug Administration (2015) Q3D Elemental impurities - guidance for industry, U. S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER), Center for Biologics Evaluation and Research (CBER). https://www.fda.gov/downloads/drugs/guidances/ucm371025.pdf. Accessed 16 January 2019.
2. Tsang KA (2010) Teething, teething pain and teething remedies. Int Dent SA 12(5):48–61. http://www.moderndentistrymedia.com/sept_oct2010/tsang.pdf. Accessed 16 January 2019
3. Cranswick N, McGillivray G (2001) Over-the-counter medication in children: friend or foe? Aust. Prescr 24(6):149–151. https://doi.org/10.1006/taap.1998.8577
4. Nielsen GD, Soderberg U, Jorgensen PJ, Templeton DM, Rasmussen SN, Andersen KE, Grandjean P (1999) Absorption and retention of nickel from drinking water in relation to food intake and nickel sensitivity. Toxicol Appl Pharmacol 154:67–75. https://doi.org/10.1006/taap.1998.8577
5. Vincent JB (2017) New evidence against chromium as an essential trace element. J Nutr 147(12):2212–2219. https://doi.org/10.3945/jn.117.255901
6. Anderson RA (1993) Recent advances in the clinical and biochemical effects of chromium deficiency. Prog Clin Biol Res 380:221–234 8456128
7. Anderson RA (1995) Chromium and parenteral nutrition. Nutrition 11(1 suppl):83–86
8. Sun H, Brocato J, Costa M (2015) Oral chromium exposure and toxicity. Curr Environ Health Rep 2(3):295–303. https://doi.org/10.1007/s40572-015-0054-z
9. EFSA Panel on Dietetic Products, Nutrition and Allergies (2014) Scientific opinion on dietary reference values for chromium. EFSA

J 12(10) https://efsa.onlinelibrary.wiley.com/doi/epdf/10.2903/j.efsa.2014.3845. Accessed 16 January 2019

10. Jurowski K, Krośniak M, Fołta M, Cole M, Piekoszewski W (2019) The toxicological analysis of Pb and Cd by ET AAS in local anaesthetics for teething (teething gels) based on herbs available in Polish pharmacies. Trace Elem Biol Med 52:18–21. https://doi.org/10.1016/j.jtemb.2018.11.005
11. Kasznia-Kocot J, Zachwieja Z, Chłopicka J, Krośniak M (1996) The content of certain trace elements and heavy metals in the hair of children from Chorzów. Pediatr Pol 71(2):31–36
12. IARC (2012) Arsenic, metals, fibres, and dusts: a review of human carcinogens. Monographs on the evaluation of carcinogenic risks to humans. International Agency for Research on Cancer, World Health Organization, Lyon, 100C. https://monographs.iarc.fr/wp-content/uploads/2018/06/mono100C.pdf. Accessed 16 January 2019
13. US EPA (1996) Nickel, soluble salts (CASRN various). Integrated Risk Information System (IRIS). https://cfpub.epa.gov/ncea/iris/iris_documents/documents/subst/0271_summary.pdf. Accessed 16 January 2019
14. Alhusban A, Ata S, Shraim S (2018) The safety assessment of toxic metals in commonly used pharmaceutical herbal products and traditional herbs for infants in Jordanian Market. Biol Trace Elem Res 187:307–315. https://doi.org/10.1007/s12011-018-1367-1