Journal of Trace Elements in Medicine and Biology 52 (2019) 18–21

Contents lists available at ScienceDirect

Journal of Trace Elements in Medicine and Biology

journal homepage: www.elsevier.com/locate/jtemb

Analytical methodology

Toxicological analysis of Pb and Cd by ET AAS in local anaesthetics for teething (teething gels) based on herbs available in Polish pharmacies

Kamil Jurowski[a,b,*], Mirosław Krośniak[c], Maria Fołta[c], Michael Cole[d], Wojciech Piekoszewski[e,f]

[a] Independent Researcher, Poland
[b] Faculty of Health Promotion, Kraków Higher School of Health Promotion, Krowoderska 73, 31-158 Kraków, Poland
[c] Department of Food Chemistry and Nutrition, Medical College, Jagiellonian University, Medyczna 9, 30-688 Kraków, Poland
[d] Faculty of Science and Technology, Anglia Ruskin University, East Road, Cambridge, United Kingdom
[e] Department of Analytical Chemistry, Faculty of Chemistry, Jagiellonian University in Kraków, Gronostajowa 2, 30–387 Kraków, Poland
[f] School of Biomedicine, Far Eastern Federal University, Sukhanova 8, Vladivostok, 690950, Russia

ARTICLE INFO

Keywords:
Atomic absorption spectrometry
Metal analysis
Teething gels
Metals impurities
Infants

ABSTRACT

Studies related to the toxicological analyses of metallic impurities in pharmaceuticals (drugs) is an important issue but there is a lack of refereed literature around the safety of teething remedies from herbal origin related to toxic metals impurities. In this article, the levels of Pb and Cd were measured in samples of local anaesthetics for teething (gels) based on herbs. This article was motivated by the fact that Pb and Cd are relevant toxic metals that may cause an adverse effect in babies even at low levels. Additional justifications were (1) the insufficient control of metal impurities in teething gels, (2) the lack of sufficient validation steps and (3) the inadequate sensitivity of applied analytical techniques. The aim of this article was a qualitative and quantitative analysis of Pb and Cd in the most popular and available local anaesthetic for teething (teething gels) based on herbs (n = 5) available in Poland. Metals were determined by ET AAS (electrothermal atomization atomic absorption spectrometry), after digestion in a microwave unit using concentrated nitric acid. It was observed that all samples contained Pb and Cd. The levels of Pb and Cd as impurities (independently of the producer and declared composition) are similar. The concentrations of Pb and Cd, at ng/day levels, to which the user is exposed at daily doses meet the standards of the directive ICH Q3D.

1. Introduction

The impurities, including the metals Pb and Cd, in pharmaceuticals (drugs) often possess unwanted toxicological effects by which any benefit from their administration may be outweighed [1]. Metal impurities in pharmaceuticals may arise from several sources, including, especially, [2]:

- residual catalysts that were added intentionally in synthesis
- metal reagent residues;
- impurities through interactions with processing equipment or container/closure systems
- components of the drug product.

Since metallic impurities do not provide any therapeutic benefit to the patient, their occurrence and levels in the pharmaceuticals (drugs) should be controlled within acceptable limits. Hence, monitoring of toxic metals in-process intermediates and final drug substances is an important activity in the modern pharmaceutical industry. There is a dearth of literature on the metal content of pharmaceutical products produced for infants. One of the most important problems are contaminations/impurities of toxic metals in teething gels.

Without a doubt, the management of teething pain is still the most frequently sought teething advice nowadays [3,4]. The timing of tooth eruption varies by as much as a half year. Most parents prefer to avoid applying pharmaceuticals during this process. A wide range of effective teething remedies based on herbs is available in pharmacies [5]. An assortment of teething agents is commonly recommended for relieving the pain and discomfort of teething [6]. The most popular teething agents are local anaesthetics (teething gels) usually based on herbs. It

Abbreviations: AAS, atomic absorption spectrometry; ET, electrothermal atomization; ICH Q3D, International Council for Harmonization 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

* Corresponding author at: Kraków Higher School of Health Promotion, Krowoderska 73, 31-158 Kraków, Poland.
E-mail address: kamil.jurowski@gmail.com (K. Jurowski).

https://doi.org/10.1016/j.jtemb.2018.11.005
Received 5 July 2018; Received in revised form 17 September 2018; Accepted 6 November 2018
0946-672X/

should be noted that the main ingredients in teething gels are: salicylic acid, lignocaine, tannic acid, menthol, thymol, glycerol, ethanol (even up to 40%) [6] and also different kinds of herbs. Some of these xenobiotics have the potential to be harmful in overdose. However, these pharmaceuticals are safe if applied as recommended [6]. Moreover, the important issue is also drug interactions when additional agents are used, such as herbal remedies, or preparations which contain high concentrations of ethanol [4]. The crucial toxicological problem can be toxic metals impurities in teething gels (local anaesthetics) based on herbs because herbs can be an important source of these contaminations.

The aim of this article was to determine the levels of Pb and Cd in local anaesthetics for teething (teething gels) based on herbs, available in Polish pharmacies. The choice of metals was justified by their potential possibility of occurrence (based on review of the literature). We analyzed the five most popular teething gels based on herbs which were available in Polish pharmacies. Pb and Cd were determined by atomic absorption spectrometer using electrothermal atomization (ET AAS). The findings are interpreted in line with directive ICH Q3D – the ICH guideline Q3D on elemental impurities published by the European Medicines Agency.

2. Materials and methods

2.1. Reagents

Demineralized water (Millipore) and concentrated nitric acid (65%) at spectroscopic grade Merck (SupraPur, Darmstadt, Germany) were used in all analytical steps. Standard solutions of Pb and Cd were prepared by dilution of certified standard solutions (1000 µg/L MERC of corresponding metal ions: $Pb(NO_3)_2$ in 0.5 mol/L HNO_3, and $Cd(NO_3)_2$ in 0.5 mol/L HNO_3).

Accuracy was evaluated by using the certified reference material: Corn Flour (INCT-CF-3) obtained from the Institute of Nuclear Chemistry and Technology Department of Analytical Chemistry.

All glass equipment and plastic materials were previously treated for 24 h in 0.5 mol/L nitric acid and rinsed with demineralized water.

2.2. Applied apparatus

For the acid digestion of samples (Section 2.5. *Sample preparation for metallic elements analysis*), a microwave oven MDS 2000 (CEM USA) and microwave-assisted digestion procedure was used.

All analyses were performed on a Perkin-Elmer 5100ZL (CT, USA) atomic absorption spectrometer with Zeeman background correction and electrothermal atomization. Measurements were made with a Cd hollow-cathode lamp of 4 mA, with the slit width set at 0.7 nm. For Pb determination, a Pb hollow-cathode lamp of 10 mA with a slit width of 0.7 nm was used. The AAS operating conditions applied in studies are presented in supporting information - Table S1.

Argon (purity: 99.99%) was applied as a purge gas. The time-temperature program of the graphite furnace for analyzed metals is shown in supporting information – Table S2. and Table S3.

2.3. Samples

The five most popular and available teething gels based on herbs were analyzed. The teething gels were chosen based on the results of paediatricians' opinions from Kraków, a questionnaire from 15 parents (27–36 years old) from Niepołomice (Poland) and a literature overview. All products were purchased in pharmacies (drugstores) in Kraków or Niepołomice (Poland). To maintain the highest methodological standards, each pharmaceutical was coded (A, B and so on). A short description of the samples (declared ingredients) is given in the supporting information – Table S4.

2.4. Sampling

A representative sample from the original packaging of each product was collected for the analyses. To avoid potential contamination during the analytical procedure, all steps of the analytical procedure were carried out in plastic equipment. Each sample was homogenized before weighing for the analysis. The first few centimetres of the gel were discarded since some products in their tubes have an aluminium lid which could also be a source of metal contamination.

2.5. Sample preparation

Microwave-assisted digestion was used to prepare samples using wet digestion, in 5.0 mL of nitric acid and about 0.3 g gel sample. The details of the microwave-assisted digestion procedure are included in supporting information – Table S5. The solutions obtained were diluted in volumetric flasks to 20 mL using demineralized water and kept as stock sample solutions at room temperature (20–25 °C) until analysis.

2.6. Pb and Cd analysis procedure

The basic workflow of the quantitative analysis of Pb and Cd in teething gels is presented in Fig. 1.

2.7. Analytical calibration and quality control approaches

The linear range of calibration function reached from the detection limit up 0.0; 1.0; 2.0; 5.0; 10.0 Pb µg/L and 0.0; 0.5; 1.0; 2.0 µg/L for Cd. The values of the correlation coefficient (R) are good indicators of the linearity for AAS instruments giving precise and accurate results [7,8]. The correlation coefficients obtained (0.998 for Pb and 0.998 for Cd) indicated that the analyses were both precise and accurate.

The recoveries were 97.0% for Pb and 96.5% for Cd. The recoveries were calculated as the quotient of the determined level and the known amount of the determined element expressed as a percentage.

The limit of detection (LOD) is defined as (3 SD)/a, where SD is the standard deviation corresponding to ten blank injections and "a" is the slope of the calibration function, obtained for each metal. The calculated LOD's were 0.45 µg/L for Pb and 0.15 µg/L for Cd. On the other hand, LOQ is defined as (10 SD)/a, where SD is the standard deviation corresponding to ten blank injections and "a" is the slope of the calibration curve, obtained for each heavy metal. The calculated LOQs were 0.91 µg/L for Pb and 0.30 µg/L for Cd.

The quality control and validation of applied methodology are confirmed by previously described studies [9,10] using the same methodology and apparatus.

2.8. Data processing

All analyses were performed in triplicate. The mean and standard deviation (SD) were calculated from these measurements. Data were analyzed using Microsoft Office Excel 2016 for Windows.

3. Results

The results for Pb and Cd can be presented according to two approaches:

1) raw results, i.e. metal per kg of gel;
2) levels of metals in one dose (about one drop of teething gel applied in one administration). These approaches are important because it is possible to obtain information about:
3) the concentrations of Pb and Cd in a concentrated product (the ability to check the fulfilment of standards);
4) the actual amount of Pb and Cd consumed in one portion (single exposure);

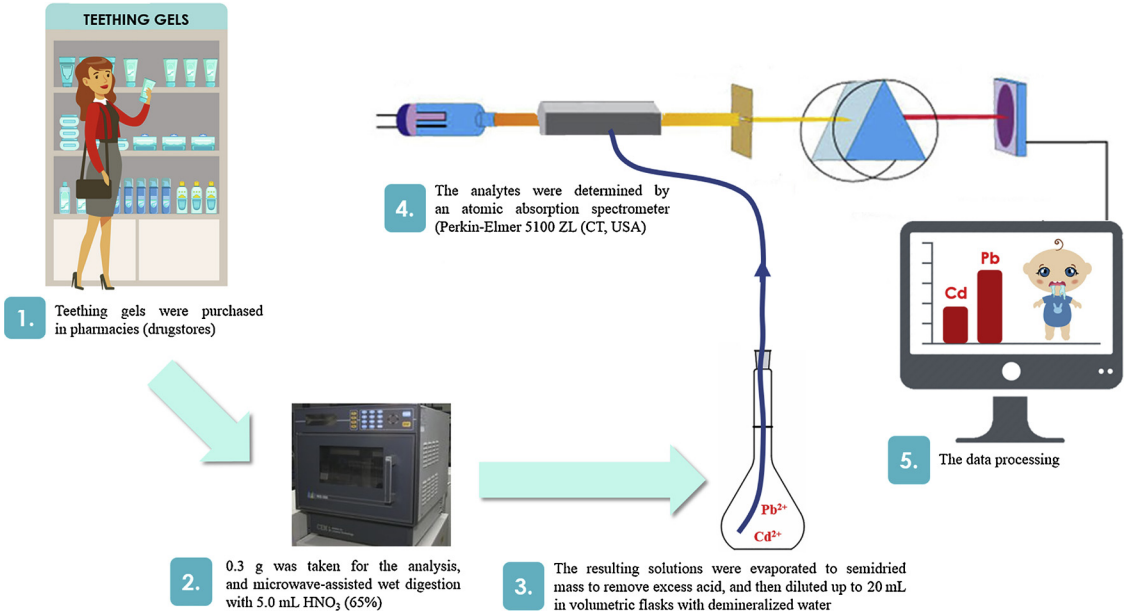

Fig. 1. The basic workflow of toxicological analysis of two toxic metals – Cd and Pb in teething gels.

5) the daily dose of Pb and Cd taking into account the maximum daily dose.

3.1. Raw results (metal per kg of mass)

The triplicate results of each composite sample of teething gel (n = 5) are given in Table 1, as μg of toxic metal per kg of gel. Pb and Cd were determined in all analyzed samples. Pb levels were highest in sample A (39.81 ± 8.41 μg/kg) and sample D (43.93 ± 3.10 μg/kg) – approximately two times higher than in other samples (18.68–21.77 μg/kg). The Cd levels were similar for all pharmaceuticals (6.98–9.90 μg/kg).

3.2. One-time administration of applied teething gels

While the raw results of Pb and Cd in the teething gels are extremely important due to the safety assessment of metal impurities in pharmaceuticals, the more valuable information from a consumer point of view is the actual level in the one-time administration of the product (in the appropriate amount of gel applied with a single administration). Based on information in the leaflet for each pharmaceutical, a drop of pea-sized gel should be applied to the painful area with a clean finger. Hence, the average volume of one drop was calculated as approximately 0.15 g of each gel. The calculated levels of Pb and Cd in the one-time administration of applied teething gels are presented in Table 2. These results are also necessary for the calculation of the daily exposure of Pb and Cd (the maximum daily dose of applied pharmaceuticals).

Table 1
The concentrations of Pb and Cd in analyzed samples (gels, μg/kg); SD – standard deviation.

Sample		Pb concentration, μg/kg		Cd concentration, μg/kg	
No.	Code	Mean	SD	Mean	SD
1.	A	39.81	8.41	7.30	0.62
2.	B	18.68	3.90	6.98	0.84
3.	C	21.24	1.77	6.99	0.03
4.	D	43.93	3.10	9.90	0.07
5.	E	21.77	5.01	8.91	0.64

Table 2
The concentration of Cd and Pb in analyzed samples (gel, ng/0.15 g) including one-time administration.

Sample		Cd concentration, ng/0.15 g	Pb concentration, ng/0.15 g
No.	Code	Mean	Mean
1.	A	1.10	5.97
2.	B	1.05	2.80
3.	C	1.05	3.19
4.	D	1.48	6.59
5.	E	1.34	3.27

3.3. Daily exposure of Pb and Cd in applied teething gels according to the maximum daily dose of applied pharmaceuticals

The application of teething gels in the infancy period is very individual and depends on many different factors. For safety assessment of this pharmaceutical type calculation of the maximum daily dose of applied pharmaceuticals is necessary. Usually, the application of each gel should be repeated three times a day (up to six times), especially after meals and before falling asleep. The daily exposures to Pb and Cd through applied teething gels were calculated considering the maximum use during the day – Table 3. The daily intake of Cd is relatively constant between gels (6.28–8.91 ng/day). On the other hand, the Pb levels were more variable ranging from 16.81 to 39.53 ng/day.

4. Discussion

Pb and Cd are metals which are significantly cumulatively toxic.

Table 3
A daily dose of Cd and Pb in analyzed samples (gel, ng/day).

Sample		Cd daily intake, ng/day	Pb daily intake, ng/day
No.	Code	Mean	Mean
1.	A	6.57	35.82
2.	B	6.28	16.81
3.	C	6.28	19.12
4.	D	8.91	39.53
5.	E	8.02	19.60

Infants may be particularly sensitive to the toxic effects of metals because they tend to absorb a higher fraction of an oral dose, and because of their developing body systems (especially the nervous system) [11]. Healthy infants are likely to receive proportionately lower doses of pharmaceutical products and, although the proposed limits apply principally to adults, they have been set at a sufficiently low level to be applicable to younger age groups [11].

It should be noted that chapter the *Heavy metals* (2.4.8) in the 9th Edition of the European Pharmacopoeia has been deleted from individual monographs on substances for pharmaceutical use, except those for veterinary use only. This was described in two press releases: 1) European Pharmacopoeia on elemental impurities (April 2015) [12] and 2) European Pharmacopoeia on elemental impurities - clarification for products outside the scope of the ICH Q3D (International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use) guideline (August 2015) [13]. This completes the first step of the European Pharmacopoeia strategy for the implementation of the Directive ICH Q3D guideline on elemental impurities. This directive aims to limit the presence of toxic metals in pharmaceutical products intended for human use [2].

4.1. Cadmium

Based on permitted concentrations for cadmium impurities in pharmaceutical products (oral concentration) recommended by directive ICH Q3D (0.5 μg/g [2]), all of the samples analyzed (see Table 1) meet the guidelines.

It should be noted that a sensitive endpoint for oral exposure to cadmium and cadmium salts is renal toxicity [14]. Additionally, skeletal and renal effects are observed at similar exposure levels and are a sensitive marker of exposure to this element [15]. Since many studies around oral exposure to Cd in rats and mice showed no evidence of carcinogenicity, the renal toxicity endpoint was used to establish the oral permitted daily exposure (PDE) for this element, i.e. 5.0 μg/day. The results of this study (see Table 3) show that all samples analyzed are characterized by results below the PDE.

4.2. Lead

Considering the concentration limits for lead impurities in pharmaceuticals via oral concentration recommended by directive ICH Q3D (0.5 μg/g [2]), all of the samples analyzed (see Table 1) meet the guidelines.

It is important that adverse neurobehavioral effects (blood lead levels < 5 μg/dL) should considered the most sensitive and relevant endpoint in humans after oral exposure for lead [16]. Taking in to account the US EPA model (Integrated Exposure Uptake Biokinetic (IEUBK) Model) [17] based on the assumption of 100% absorption (no other sources of lead), oral intake of 5 μg/day translates into a blood level of 1–2 μg/dL for children age 0–7 years. Hence, PDE for this element should be taken as 5.0 μg/day. The results of this study (see Table 3) shows that all analyzed samples are characterized by results below PDE.

5. Conclusions

The measured levels of Pb in teething gels avaiable in Polish Pharmacies range from 16.81 μg/kg to 39.53 μg/kg. The levels of Cd range from 6.28 to 8.91 μg/kg. It can be assumed that the Pb and Cd levels in all of the gels tested occur at a very low level (gel, μg/kg). Their content in a single administration is also very low (0.15 ng/g) and is not a threat to infants. None of the teething gels analyzed represents a health hazard to the infants. Moreover, the levels of Cd and Pb meet the standards of directive ICH Q3D. Hence, considering the daily dose of Pb and Cd (gel, ng/day), the results are satisfactory and confirm the safety of teething gels for infants. It would be valuable to carry out a broader study considering other teething medicaments containing herbs (for example from other countries) to build upon this data.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jtemb.2018.11.005.

References

[1] N.R. Rao, M.K. Talluri, An overview of recent applications of inductively coupled plasma-mass spectrometry (ICP-MS) in determination of inorganic impurities in drugs and pharmaceuticals, J. Pharm. Biomed. Anal. 43 (1) (2007) 1–13, https://doi.org/10.1016/j.jpba.2006.07.004.
[2] 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), 2015 September https://www.fda.gov/downloads/drugs/guidances/ucm371025.pdf.
[3] A. Owais, F. Zawaideh, O. Bataineh, Challenging parents' myths regarding their children's teething, Int. J. Dent. Hyg. 8 (1) (2010) 28–34, https://doi.org/10.1111/j.1601-5037.2009.00412.x.
[4] A.K. Tsang, Teething, teething pain and teething remedies, I. Dent. S. A. 12 (5) (2010) 48–61 http://www.moderndentistrymedia.com/sept_oct2010/tsang.pdf.
[5] G.T. McIntyre, G.M. McIntyre, Teething troubles? Br. Dent. J. 192 (5) (2002) 251–255, https://doi.org/10.1038/sj.bdj.4801349a.
[6] N. Cranswick, G. McGillivray, Over-the-counter medication in children: friend or foe? Aust. Presc. 24 (6) (2001) 149–151 https://www.ajol.info/index.php/cme/article/viewFile/43868/27387.
[7] Q. Duan, H.V. Gupta, S. Sorooshian, A.N. Rousseau, R. Turcotte, Calibration of Watershed Models, first ed., American Geophysical, Union, Washington, 2004.
[8] T. Wagener, H.S. Wheater, H.V. Gupta, Identification and evaluation of watershed models, in: Q. Duan, H.V. Gupta, S. Sorooshian, A.N. Rousseau, R. Turcotte (Eds.), Calibration of Watershed Models, first ed., American Geophysical, Union, Washington, 2004, pp. 29–47.
[9] H. Barton, Predicted intake of trace elements and minerals via household drinking water by 6-year-old children from Kraków, Poland; Part 2: cadmium, 1997–2001, Food Addit. Contam. 22 (9) (2005) 816–828, https://doi.org/10.1080/02652030500164441.
[10] H. Barton, Z. Zachwieja, M. Folta, Predicted intake of trace elements and minerals via household drinking water by 6-year-old children from Krakow (Poland); Part 1: lead (year 2000), Food Addit. Contam. 19 (10) (2002) 906–915, https://doi.org/10.1080/02652030210145919.
[11] Guideline on the Specification Limits For Residues of Metal Catalysts or Metal Reagents, European Medicines Agency, London, 2008 21 February Doc. Ref. EMEA/CHMP/SWP/4446/2000 http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500003586.pdf.
[12] PH. EUR, Policy on Elemental Impurities - Clarification for Products Outside the Scope of the ICH Q3d Guideline, Strasbourg, France, 7 April (2015) https://www.edqm.eu/sites/default/files/press_release_elemental_impurities_pheur_2015.pdf.
[13] PH. EUR, Policy on Elemental Impurities - Clarification for Products Outside the Scope of the ICH Q3d Guideline, Strasbourg, France, 7 August (2015) https://www.edqm.eu/sites/default/files/press_release_pheur_policy_on_elemental_impurities_august_2015.pdf.
[14] J.P. Buchet, R. Lauwerys, et al., Renal effects of cadmium body burden of the general population, Lancet 336 (8717) (1990) 699–702 PMID: 1975890.
[15] O. Faroon, A. Ashizawa, S. Wright, P. Tucker, K. Jenkins, L. Ingerman, C. Rudisill, Toxicological Profile of Cadmium, Agency for Toxic Substances and Disease Registry, Atlanta, (2012), pp. 273–274 PMID: 24049863.
[16] NTP Monograph on Health Effects of Low-level Lead, Office of Health Assessment and Translation, Division of the National Toxicology Program, National Institute of Environmental Health Sciences, National Institutes of Health, U.S. Department of Health and Human Services, 2012 June 13 https://ntp.niehs.nih.gov/ntp/ohat/lead/final/monographhealtheffectslowlevellead_newissn_508.pdf.
[17] K. Hogan, A. Marcus, R. Smith, P. White, Integrated exposure uptake biokinetic model for lead in children: empirical comparisons with epidemiologic data, Env. H. Perspec. 106 (Suppl 6) (1998) 1557–1567 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1533426/.