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RESEARCH ARTICLE

MERCURY DETERMINATION IN DRUG AND COSMETIC PRODUCTS

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ABSTRACT

Mercury and its chemical compounds are considered very toxic for plants and human beings. Nevertheless, mercury compounds are used in cosmetic and drug products, and particularly in whitening creams in Eastern countries. Mercury is used as a preservative (antifungal and antiseptic) in pharmaceutical liquid or cremates products, even in eye, ear and nasal drops. Creams containing mercury have been manufactured and used despite their confirmed health risks. These products are widely available in pharmacies and beauty aid stores in Albania. They are primarily used by women for makeup and skin lightening effects and also for local pharmaceutical treatments in the human body. The aim of this work was to analyze the mercury content in some cosmetic and drug products by using the cold vapor atomic absorption technique (CVAAS). Preliminary studies were conducted in order to evaluate a proper digestion method able to destroy the matrix of the sample and to convert all forms of mercury to elemental mercury. The results showed that a mixture of H_2SO_4 and HNO_3 digestion provided a higher signal of mercury by destroying first the matrix of the cream type samples. Microwave digestion of liquid pharmaceutical products in the presence of strong acids (HNO_3) and strong oxidants ($K_2Cr_2O_7$) was established as an effective method for a complete digestion of the liquid samples. A total of 19 samples from the local pharmacies and beauty shop market were investigated. No warning information was noted on the packaging of these products. The mercury content of six of them varied between 18.5-0.10 ppm (mg/L or mg/kg). The results are compared with the well known regulations regarding the limit of mercury content for each category of the samples. Even so, the products containing mercury are poorly controlled in Albania.

INTRODUCTION

The toxicity of some mercury compounds as well as their ability to be absorbed through the skin has been long understood. Interestingly, mercury has been used as a skin bleaching agent and as a preservative in pharmaceutical and cosmetic products for many years. Mercury is used as a preservative (e.g., thimerosal, as an antifungal and antiseptic agent) in many drug products such as vaccines (influenza, hepatitis B, DPT), ointments (eye ointments, hemorrhoid ointments), contact-lens solutions and ear, eye and nasal drop solutions. Currently mercury can be present in pharmaceutical products even when it is not listed on the label or on the product information leaflet. It is usually introduced as a preservative or anti-microbial in the form of thimerosal. Thimerosal or merthiolate is a derivative of thiolsalicylate where ethyl-mercury is attached though the sulfur or the thiol group, and its half molecular weight belongs to mercury. Thimerosal has been and is used in a variety of drug products especially for local use, because of its antiseptic properties. These products include vaccines, cosmetics, eye, ear and nasal drops and saline solutions. In the human body mercury is metabolized or degraded in ethylmercury (C2H5Hg+) and thiosalicylate (Center for Biologics Evaluation and Research 2008). The allergic reaction that he caused may be a simple conjunctivitis or a dermaconjuctivitis with the participation of the eyelids. The EU guidelines are specifying that Thimerosal must not be listed any more as a preservative agent in drug products because of its allergic and very toxic properties (http://www.fda.gov/BiologicsBloodVaccines/Vaccines/QuestionsaboutVaccines/UCM070430).

It is found that the women who use mercury-containing creams have elevated mercury levels in their hair, blood and urine (Kahatano *et al.,* 1998; Glahder *et al.,* 1999). Long-term use of mercury-based skin lighteners often produces a characteristic slate gray skin color (Luderschmidt and Plewig 1979), and kidney damage (Li *et al.,* 2010; Oliveira *et al.,* 1987). Mercury is also transferred from a mother to her nursing infant in breast milk (Bose-O'Reilly *et al.,* 2008). Mercury in such preparations can enter the human body by skin absorption (Bourgeois *et al.,* 1986; De Bont *et al.,* 1986). Their use has been associated with renal (Oliveira *et al.,* 1987) neurological (Dyall-Smith and Scurry 1990; Smith *et al.,* 1994; Centers for

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Disease Control and Prevention (CDC) 1996) and dermal (Dyall-Smith and Scurry 1990; Smith *et al.,*1994; Tlacuilo-Parra *et al.,* 2001) toxicity. FDA regulatory guidelines regard a cosmetic's mercury concentration as safe at less than 1 ppm for products used around the mouth, or less than 65 ppm for products used around the eye (http://www.law.cornell.edu/cfr/text/21/700.13 Accessed date 29.04.2014), while no stricter regulations exist about pharmaceutical products. On the other hand, mercury content exceeding 10 ppm is a risk for pregnant women and children (US EPA 2001; http://www.atsdr.cdc.gov/es/phs/es_phs46.html, Harada *et al.,* 2001; Lauwerys *et al.,* 1978; WHO 1990; WHO 1991; Bonnie *et al.,* 1987). The skin creams and drug products such as eye drops solution and ointment, nasal and ear drops solution containing mercury are available in the market and pharmacies in Albania. The label of most products does not specify their ingredients, so the consumer does not have any choice for selecting suitable products. No safety regulations for cosmetics and other products that contain mercury or mercury compound exist in Albania. Due to the uncontrolled exposure, cosmetic products should be thoroughly evaluated for safety before marketing. It is often difficult to determine whether a drug contains mercury. Ecology recommends you require your vendors to identify all mercury containing products and provide mercury free formulations when they are available. Usually medical personnel do not have adequate information to designate mercury containing pharmaceuticals without testing.

Several analytical techniques and methods are used for the determination of mercury in different samples and matrices, that provide a high selectivity and precision, like spectrophotometric methods. (Rathje 1969), AAS and CVAAS methods (Lazo and Cullaj 2002; Lazo and Kucuku 2012; Ferrat *et al.,* 2002; Cappon and Smith 1997), cromatografic methods (Shank *et al.,* 1962). The atomic absorption methods are defined as the methods with high precision and sensitivity in the determination of mercury. In this study we worked for the optimization of the method for the determination of mercury in cosmetic and drug products. The method used was the method of the atomic absorption combined with cold-vapor, CVAAS (Cold-vapor atomic absorption spectrometry). CVAAS method is often used in the determination of trace mercury levels, as routine tests for determination of mercury in different products. (Lazo and Cullaj 2002; Lazo and Kucuku 2012; Ferrat *et al.,* 2002; Shah *et al.,* 2010). Several analytical techniques described the determination of mercury in different environmental and biological samples (Lazo and Cullaj 2002; Lazo and Kucuku 2012), while the matrix of pharmaceutics and cosmetics are not simple. They contain many ingredients and often requires time-consuming for sample treatments. In the present study, we determined mercury content by cold vapour atomic absorption technique (CVAAS) in different brands of pharmaceuticals cream and face cream samples. These samples were collected from various pharmacies and beauty aid stores in the market of Tirana in order to check their safety and provide the evidence of potential exposure to mercury poisoning.

MATERIALS AND METHODS

9 medical samples and 10 cosmetic samples were purchased in a range of stores that included pharmacies, department stores and regional chain stores. Samples consisted of pharmaceutical liquid and cream products and cosmetics from very "low-end" brands to very "high-end" brands with the majority being the most widely available, common brands.

Reagents

The modified EPA method 245.5 (U.S. EPA, 1993) have used the direct additions of the concentrated H_2SO_4 and HNO_3 and 1 ml of a 5% solution of $K_2Cr_2O_7$ (5g $K_2Cr_2O_7$ w/v was prepared in 100 ml of DI water). The reducing reagent was a 15% solution of stannous chloride dihydrate w/v in 7 % v/v HCl. A 2.5 mg/L working mercury standard was prepared from serial dilutions of a 1000 mg/L mercury standard (Merck). Each serial dilution was prepared with 3% HCl v/v.

Mercury measurement system

Instrument Settings

The measurements were carried out using a model Varian 10+ AAS spectrometer equipped with a home-made cold-vapor system (Lazo and Cullaj 2002). CVAA instrument analysis was performed for mercury determination. The system used for volatilization and atomization of mercury and the operation of the cold vapor system is described in our previous publications (Lazo and Cullaj 2002; Lazo and Kucuku 2012). The optimum conditions for the operation of the cold vapor system were: volume of sample injected: 1 to 20 ml and flow rate of air carrier gas: 2.0 L/min.

A mercury hollow cathode lamp operated at 5 mA was used as the radiation source. Measurements were carried out in the absorbance peak high mode at 253.7 nm, using a spectral bandwidth of 0.5 nm and Deuterium lamp for background correction.

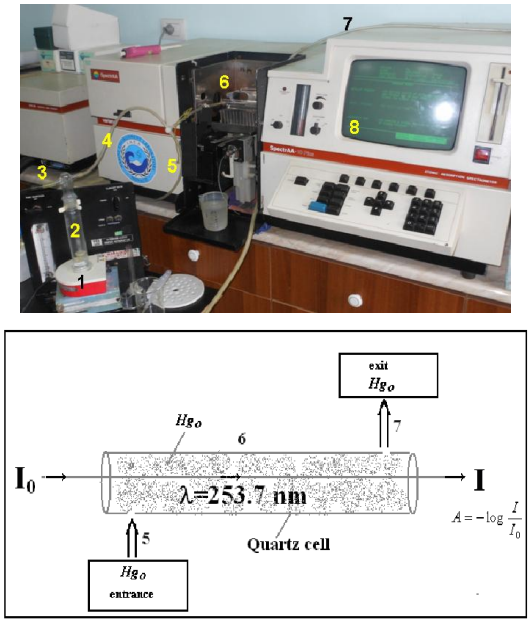

Figure 1. The picture of Varian 10+ instrument and home-made could vapor system for mercury determination

1. Magnetic stirrer; 2. Reaction flaskt; 3. Air inlet in reaction flaskt; 4. Air outlet from reaction flask; 5. Air flow washing tube; 6. Quartz cell; 7. Outlet of quartz cell; 8. Monitor

Digestion Procedure

CVAAS method for the determination of Hg in samples with organic matrices (such as cosmetic and drug products are) passed throw two important steps (Lazo and Cullaj 2002; Lazo and Kucuku 2012; Ferrat *et al.,* 2002; Cappon and Smith 1997; Morita *et al.,* 1998; Chen *et al.,* 2002; Kelly *et al.,* 2006):

1. The chemical treatment of the sample in order to transform different forms of mercury present in the sample in inorganic mercury (Hg^{2+}) through the digestion and oxidation of the sample.
2. The reduction of (Hg^{2+}) ions in its atomic form (Hg^{0}).

Different digestion methods are applied. For the destruction of the organic matrices of the samples (Lazo and Cullaj 2002; Lazo and Kucuku 2012; Cappon and Smith 1997; Kelly *et al.,* 2006). The work of this study is focused on the determination of mercury in cosmetic and drug products after the optimization of two important steps of the analysis:

1. The process of sample digestion with microwave or with the half pressure Teflon tubes that is able to transform the organic mercury in inorganic mercury Hg^{2+}. Sample digestion is a critical step in the destruction of the organic matrices, because the presence of organic compounds can lead to the recreation of the organometalic compounds of Hg^{2+} and in this way can prevent the determination of mercury with CVAAS method. The modified EPA method 245.5 (U.S. EPA, 1993) is used as a reference procedure for sample digestion that is modified in our lab to access total digestion of the samples.
2. The process of the determination of mercury by using the CVAAS method.

The optimization of the digestion procedure

Cream samples

The pharmaceutical and cosmetic cream samples were weighed (0.2 to 0.5 g) into the Teflon half pressure digestion tubes. The samples were predigested with 2 ml of H_2SO_4 and 2 ml HNO_3. The concentrated acids were added to the digestion tubes, the tubes were loosely capped and the samples were predigested in room temperature overnight than the temperature was increased at 80° C for 2 hours. This initial predigest step dissolved and dispersed the samples. After the samples were dissolved and allowed to cool to room temperature, 5 ml $K_2Cr_2O_7$ 5% solution and 5 ml 3% HCl was added to each digestion tube. The tubes were loosely closed, swirled then heated at 200° C for 2 hours. After again cooling to room temperature, the digestion tubes were then brought up to a final volume of 50 ml with 3% HCl with thorough mixing prior to CVAA analysis of the samples.

Liquid samples

MDS-6 microwave was used for the digestion of liquid samples by modifying US-EPA 3052 standard method in combination with the digestion method given in the instruction manual of MDS-6 microwave for biological, botanical and oil samples (see Table 1).

Table 1. The reagents used for microwave digestion of different samples (US-EPA 3052)

Type of samples	Reagents and their volume (ml)			
	HNO_3	HF	HCl	H_2O_2
Biological	9	0	1	2
Botanical	9	0	0.5	2
Oil samples	9	0.5	0.5	2

The procedures shown in Table 2 were modified by adding 0.5 ml 5% $K_2Cr_2O_7$ solution at each one. The digestion was performed in four steps at low temperature in MDS-6 microwave system.

Table 2. The MDS-6 microwave digestion program

Step	P (MPa)	t (min)	W (vat)
1	0.3	10	400 (1)
2	0.6	0.5	600 (2)
3	0.9	0.2	600 (2)
4	1.2	10	400 (1)

Septobore was used to check the recovery of the method after microwave digestion. The modified method of biological samples resulted as the best one with recovery of 98.9% calculated as an average value of 3 digested aliquots from the same flacon.

Calibration

Working mercury standards were prepared from serial dilutions of a 1000 mg/L mercury stock solution. From the final working standard of 2.5 mg/L a series of 5 standard solutions were transferred into the mercury reaction flask containing 2 ml concentrated H_2SO_4 and 5 ml 15% $SnCl_2$ solution. The blank solution was prepared in the same way as the standard solutions. The calibration plot obtained by linear regression of the absorbance against the absolute content of Hg2+ added in reaction flask (0.05 to 1.0 µg Hg^{2+}). The measurements were done at 253.7 nm and deuterium lamp was used for background correction. Peak height was measured for 3 replicates after registering the absorption curve as is shown in Fig. 2.

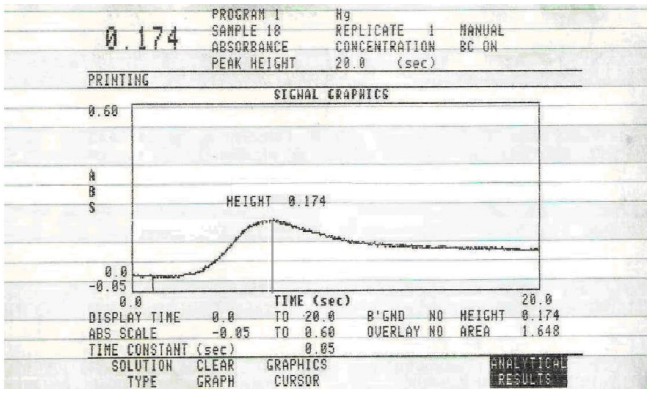

Figure 2. The signal graph of a 0.25 µg/L Hg Peak Profile

Prior to calibration of the system, peak profiles of the 0.25 µg/L Hg standard solution and the calibration blank were performed to ensure method parameter settings were optimal and would yield precise results.

The calibration curve obtained through the CV-AAS measurements of the serie of standard solution show a good linearity and high sensitivity. The range of the linear calibration curve was 0.05–1.00 µg/L Hg content in the reaction flask (or 2.0 – 20 µg Hg). The equation was Abs = 0.0069 + 0.689x with high value of the coefficient of linearity (R^2=0.9995). The relative standard deviation (RSD) calculated from 10 successive measurements of 2.5 µg Hg content in the reaction flask standard solution, was 2.4%. The detection limit (LOD) calculated on the basis of three times the standard deviation (3σ) of ten repetitive measurements of the blank solution divided by the slope of the calibration curve is 0.005 µg/L Hg and LOQ = 10LOD = 0.05 µg/L Hg.

Quality control of the analysis

Due to the unavailability of certified material for pharmaceutical and cosmetic product analysis in our lab, the accuracy of the method was checked by using the standard addition method that was applied in a pharmaceutical sample and a cosmetic sample. The mercury content declared at the certificate provided by the producer in the pharmaceutical sample was considered as the "true" value against which was tested for the significant differences (see Table 4) of analytical result. The corresponding average mercury content given in the product leaflet is considered as a "true" content. Three samples of septobore (Ho = µ =6.73 µg/ml declared in the leaflet of the product) considered as "reference material" were analyzed by two different digestion methods as is shown in Table 3.

Table 3. Calculated data for the pharmaceutical sample septobore for two different digestion methods

Method of digestion	[1]Microwave without additional $K_2Cr_2O_7$			[2]Microwave + $K_2Cr_2O_7$		
Samples (septobore)	1	2	3	1	2	3
Hg (µg/ml)	5.01	4.92	5.39	6.45	6.44	6.54

The statistical treatment of the calculated results for the pharmaceutical sample (septobore) are shown in Table 4.

Table 4. Statistical treatment of the data (N=3)

	Hg[1]	Hg[2]
Mean	5.11	6.48
Median	5.01	6.45
Standard Deviation	0.25	0.055
Sample Variance	0.06	0.003
Minimum	4.92	6.44
Maximum	5.39	6.54
Count	3	3
Confidence Level (95.0%)	0.62	0.137
RSD (%)	4.89	0.85
Bias	1.62	0.25
H_o[3] (µg/ml)	6.73	6.73

[3] The value given in the leaflet of the product

Starting from the statistical treatment data we can see that the microwave digestion method with $K_2Cr_2O_7$ 5% as an oxidant has shown better results than the method without $K_2Cr_2O_7$ 5%. This means that the addition of $K_2Cr_2O_7$ improves the method of the digestion of mercury and gives a better definition of the digestion method of mercury and also gives an accurate determination of the amount that is in the sample. The accuracy of the digestion procedure of cream or gel samples without any description regarding the mercury content was evaluated through recovery studies by using standard addition method. The quantity of standard solution added is calculated as absolute weight of Hg given in µg (see Table 5). Five portions of 0.5 g cream sample (Canesten) was sent to the half pressure Teflon tubes and in 4 of them was added 0.050 ml of MeHg standard solution with concentration 2.5 µg/ml Hg was added. The samples are digested by heating in the presence of the mixture of acids (H_2SO_4 and HNO_3) and $K_2Cr_2O_7$ as is described for cream samples. The results of the analysis are shown in Table 5:

Table 5. The study of the recovery of the method (Canesten, m=0.5 g sample)

ΔC (µg)	$C_{eksperimentale}$ (µg)	ΔC_{eksp} (µg)	η%
0	0.158	0	-
0.125	0.273	0.121	96.8
0.125	0.269	0.128	102.4
0.125	0.283	0.131	104.8
0.125	0.279	0.126	100.8

The values of the recovery of the method, studied by means of standard addition method, are very high (96.8-104.8%), while the recovery average results η%=101.2%, or RSD=1.2%, which shows a high precision of the analysis method.

RESULTS AND DISCUSSION

Mercury Content

Most of the samples collected did not show any declaration on the packaging that they contain mercury or mercury compounds. The results of the mercury determination of the pharmaceutical and facial cream samples are shown in Table 6.

Table 6. The content of mercury in drug products for local use

Samples	Utilization	Hg (ppm)
Tetramil	Eye drops	18.50±0.26
Septobore	Eye drops	6.38±0.03
Betabioptal	Eye drops	4.62±0.14
Antibioptal	Eye drops	3.50±0.22
Polydexa	Ear drops	3.16±0.15
Operil	Nasal drops	1.31±0.13
Galsud	Nasal drops	1.94±0.05
Canesten	Antibiotic skin cream	0.15±0.016
Bivacin	Antibiotic skin cream	0.32±0.019
Hand Frost Cream (Chinese product)	Cosmetic product	0.10±0.012
Lubrider cream	Cosmetic product	0.08±0.015
Glicerine Avon	Cosmetic product	0.13±0.018
NIVEA cream	Cosmetic product	0.16±0.020
SODALCO liquid soap	Cosmetic product	0.18±0.018
Lobello (red lipstick)	Cosmetic product	1.04±0.10
Eosan gel	Cosmetic product	0.21±0.023
Colistar (red lipstick)	Cosmetic product	0.27±0.022
Ordiner red lipstick	Cosmetic product	0.23±0.015
Lens Cleaner	Cosmetic product	0.95±0.094

As can be seen, the mercury content of all analyzed samples was higher than the detection limit of CVAAS method (0.01 ppm). The highest Hg content was found in tetramil drug product (18.5 ppm). However, the mercury content of this product is lower than the FDA regulations. The mercury content found in Lobello (red lipstick) is more or less equal to FDA regulations.

Conclusion

The benefits of the digestion of the sample in a short time, the low consumption of acids and the high efficiency of the digestion used in the method with microwave make this method preferably in comparison with the classic digestion method. Microwaves have a good quality and a high performance in the preparation of different samples. Despite all the benefits that the digestion method with microwave has, there are also some flawed in the use of some reagents that cannot be used in the microwave like creams or gel products of organic compounds. Classic digestion method by using H_2SO_4 for destroying organic matrices of the samples, followed by the oxidation process in the presence of strong oxidants (HNO3 and $K_2Cr_2O_7$) provides a good quality and a high performance in the preparation of this category of samples for CVAAS analysis of mercury. Determination of mercury is difficult since it enters in the group of elements that vaporize easily. During the optimization of the digestion method in the microwave in order to achieve a better quality and precision of the analysis, it was concluded that the best method was the one that add as an oxidant $K_2Cr_2O_7$ 5%, which gave a high efficiency of analysis with RSD% <5% and the recovery of the method results in the mass of 96.8 – 104.8%, this guarantees a complete determination of mercury present in the sample.

This work could contribute to the reduction in the lack of regulatory inspection and to urge authorities to establish a control in Albania. The need of a regulatory inspection is mandatory in Albania.

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