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ORIGINAL PAPER

Evaluation of mercury content in combustible tobacco products by employing cold vapor atomic absorption spectroscopy and considering the moisture content: a comprehensive study

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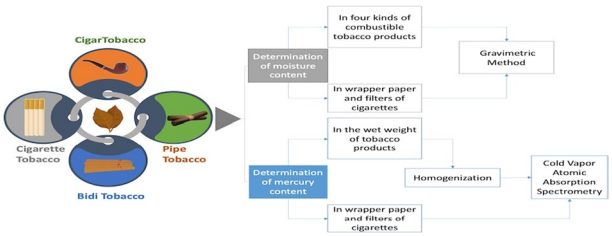
Abstract

Plants are mainly made up of water, which constitutes between 80 and 90% of their weight. Moisture factor comes across as one of the most important in tobacco products. Rapid determination of moisture content in tobacco products comes at necessity in any tobacco management plants (before and after production). Therefore, the concern has been raised in this study to evaluate the moisture content in four kinds of combustible tobacco products using the gravimetric method. In addition, a total mercury content using cold vapour atomic absorption spectroscopy has been evaluated in all chosen combustible tobacco products in this study. Determining moisture content in four types of tobacco products does not show significant differences within a product group. Moisture content ranged from 7.9% in bidis to 25% in pipe tobaccos. Mercury content in tobacco ranged from 13 µg/kg to 32 µg/kg while in cigarette wrapper paper and filter from the limit of detection (LOD) < 1.3 µg/kg to 8.2 µg/kg. As evidenced, cigarette wrapper paper and filters alone are unlikely to be a significant source of consumer exposure to mercury. However, the proposed sample preparation method provides good results for the preparation of specific material, such as tobacco products.

Graphical abstract



Keywords Cigarette · Heavy metals · Mercury · Moisture content · Tobacco · Contamination

Introduction

Nicotiana tabacum L. has been cultivated and used by mankind for over 5000 years (in various forms, e.g., combustible tobacco products, snuff, infusion, chewing products, etc.), but for most of that time, it was limited only to the American region and native Americans [1]. The discovery of tobacco by Europeans initiated a process of scientific investigation of its properties. The discovery eventually turned in consequences as a harmful addiction. Therefore, in the last few decades, there has been a tendency to promote smoking cessation [1]. One of the well-known adverse health effects of tobacco smoking is lung cancer. However, overall global tobacco consumers were 1.337 billion in 2018 according to WHO Global report on trends in prevalence of tobacco use 2000–2025 [2].

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In the market, the availability of tobacco products is categorized into two types: smokeless and combustible tobacco products. Historically, combustible tobacco products are considered one of the cult tools and products with a health benefits [1]. As its name implies, high temperature, and especially combustion, is needed to consume combustible tobacco products [3]. Combustible tobacco products can reach temperatures as high as 950 °C, as explained by Mallock et al. [3].

Unfortunately, researchers often seem to forget how complex the subject of the combustible tobacco products typology is. A broad spectrum of combustible tobacco products should be supported by an equally broad analysis. Combustible tobacco products differentiate based on the types of products, countries of origin and methods of production [4, 5]. The names of the same tobacco products are described in an inconsistency way, whereas it could be deceptive to readers. The specification and characteristics of tobacco products are also considered prior, such as used materials and methods for manufacturing the products. There is, therefore, a selective interest among researchers in cigarettes, so in this research, it was decided to examine a broader spectrum of tobacco types in order not to discriminate against the exposure of their consumers.

Combustible tobacco products can be distinguished based on how they are manufactured, materialized and their way of consumption, which includes products, such as bidis, cigarette tobaccos, cigar tobaccos, and pipe tobaccos [6, 7]. Cigarettes are, in general, one of the most popular combustible tobacco goods. The world production of cigarette products reaches thousands of tons and this proves a large group of consumers [8]. Consumer exposure to mercury in cigarette smoke is directly related to the presence of this element in cigarettes as such. These, however, are made of three components. In general, the most important one is certainly the tobacco. However, the paper wrapper is also burnt and the high temperature affects the filter as well. The latter is made of "cotton-like" plastic—cellulose acetate [9]. In contrast, other combustible tobacco products, such as cigars and pipe tobaccos made of only tobacco leaves, bidis are wrapped with tendu leaves [10].

The moisture content is one of the crucial properties of tobacco due to its influence on the brittleness of the products. It is almost impossible to smoke a dry cigar. Moisture content must be maintained in tobacco products from the time when tobacco leaves are harvested until they reach end consumers. The mentioned parameter differentiates in variety of tobacco products to maintain the quality of products, such as cigars, cigarettes, pipe tobaccos, or bidis. The sealing process of any tobacco products mostly influence the moisture content and other parameters, such as volatile component [11]. Humidity must be maintained during the transportation process from an industrial warehouse to commercial inventory.

Recently revised standard, ISO 6488:2021 specifies a procedure of water content determination by the Karl Fischer titration [12]. It is applicable to raw tobacco as well as final products within the moisture ranging from at least 2% up to 55% [12].

The gravimetric methods are another possible approach for determining the moisture content of materials. Gravimetric methods provide an advantage over Karl Fischer titration as they do not require any reagents and there is no risk of side reactions [13]. Coulometric Karl Fischer titration is more suitable for samples containing small amounts of moisture content and larger amounts may overwhelm the reagent capacity and yield false results [13, 14]. Moreover, Karl Fischer titration method needs the material to be more accurately homogenized to perform the analysis.

Mercury is one of the substances present in combustible tobacco products. The toxicity of mercury has been proven and widely described [5, 15]. Exposure to Hg can result in numerous diseases depending on the degree of exposure and the route of administration [16]. Moreover, the high toxicity of Hg is observed even at its low doses [17]. Mercury vapours are particularly poisonous due to their ability to bioaccumulate in body tissues [18]. Elemental contamination of cigars (little cigars which are also sometimes called filtered cigars) is well explained by Fresquez et al. [19]. The plant material constituting the first link in the chain deserves special attention in this context, as there are known cases of using protective preparations containing Hg. It has also been noticed that organic material, such as tobacco, usually contains mercury in form of organic compounds [18].

Another crucial issue of combustible tobacco products toxicity is the interaction of toxins, elements and compounds between tobacco and the environment. Regardless of their type, combined toxicity effects are, therefore, being observed [20, 21]. Thus, although the concentration levels of individual constituents of tobacco smoke may be nonlethal, they must be placed in the context of the high environmental pollution burden worldwide [22]. We can distinguish three types of such combined toxicity effects: synergistic, antagonistic, and additive [21]. There is evidence of greater than just additive effects seen in people coexposed to tobacco smoke and arsenic [20]. This applies to both: active and passive smoking [20]. It might be considered to call this interaction a synergistic effect [23].

One of the goals of this study was to evaluate the moisture content in a variety of combustible tobacco products using the gravimetric method. The gravimetric method was employed to determine the moisture content in combustible tobacco products due to its high efficiency over other methods. The presented study could show the proper approach for the manufacturers to evaluate moisture content during

the storage period of tobacco products. In addition, another aim of the study was to evaluate the total Hg content in a variety of combustible tobacco products. This study provides the proposal that total mercury content can be determined based on dry weight and evaluating consumer exposure to chosen combustible tobacco products. A similar approach was proposed by Milatou et al. [24] to evaluate total mercury content in tuna fish samples. The change applied in this study concerned the used drying method. A laboratory dryer was employed instead of lyophilizator used by Milatou et al. [24] This study fulfils the need for the study on the total Hg content in various combustible tobacco products and combustible tobacco products that are rarely studied for total Hg content and moisture content, such as pipe tobaccos and bidis, to determine consumer exposure.

Results and discussion

Tobacco moisture is one of its crucial properties which is important for specific product preparation processes [25]. The used gravimetric determination method of moisture content provided an advantage over the homogeneity of the material. The obtained results are presented in Fig. 1. In the case of the pipe tobacco, since the lower quartile is at 20.5% moisture content, about 75% of the pipe tobacco consists of more than 20.5% of moisture content. Moisture content in pipe tobaccos is significantly higher than in the other group of products.

It can be clearly seen in Fig. 1, in the case of cigar tobacco, where 50% of the cigar tobacco contains more than 14% of moisture. Cigar tobacco products are more variable in moisture content, which could be seen from the long upper whisker which means that the moisture content in cigar tobaccos are varied amongst the least positive quartile. While very similar moisture content for the most positive quartile.

The obtained results for cigarettes and bidis show that moisture content in both products are highly precise. Especially in the case of bidis, it has been observed that all the products showed similarity in means of moisture content.

On the other hand, the moisture content is also determined in the five of different branded cigarette products (separately in wrapper paper and filters) as well as total mercury content. Each contained materials from nine cigarettes randomly selected from the package. For each brand of cigarette, nine cigarettes were taken into account to differentiate filters and wrapper paper. It is worth mentioning that their weights are significantly lower in comparison to tobacco weight in each cigarette. Determined moisture contents in cigarette filters and wrapper paper are shown in Fig. 2.

As can be seen, cigarette filters have significantly higher water content than wrapper paper. It would be impossible to determine mercury contents in undried samples of these components. Performing analysis from dry samples and subsequent conversion of the concentration results to wet weight concentrations is the desired approach. Although presenting the results in units of dry weight concentrations does not generate any amendments and still allows comparison of results between samples, it limits the ability to estimate consumer exposure to mercury. Combustible tobacco products contain a certain amount of water when they are consumed. Therefore, the moisture content should always be considered while estimating consumer exposure.

Toxin distribution from tobacco (e.g., nicotine) has been proven to be moisture-dependent [26]. Although manufacturers process products under specific moisture conditions, the moisture content begins to vary with ambient temperature and humidity as soon as the package is opened. As a result, the ISO 3402:1999 standard specifies

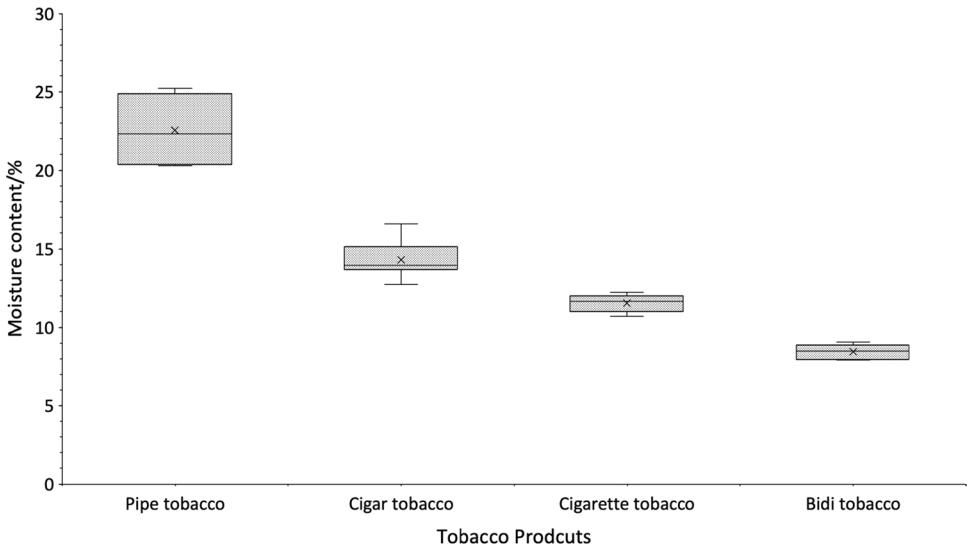

Fig. 1 Box chart of the determined moisture content in four kinds of CT tobacco products (pipe tobacco (4 brands), cigar tobacco (34 brands), cigarette tobacco (5 brands), and bidi tobacco (5 brands))

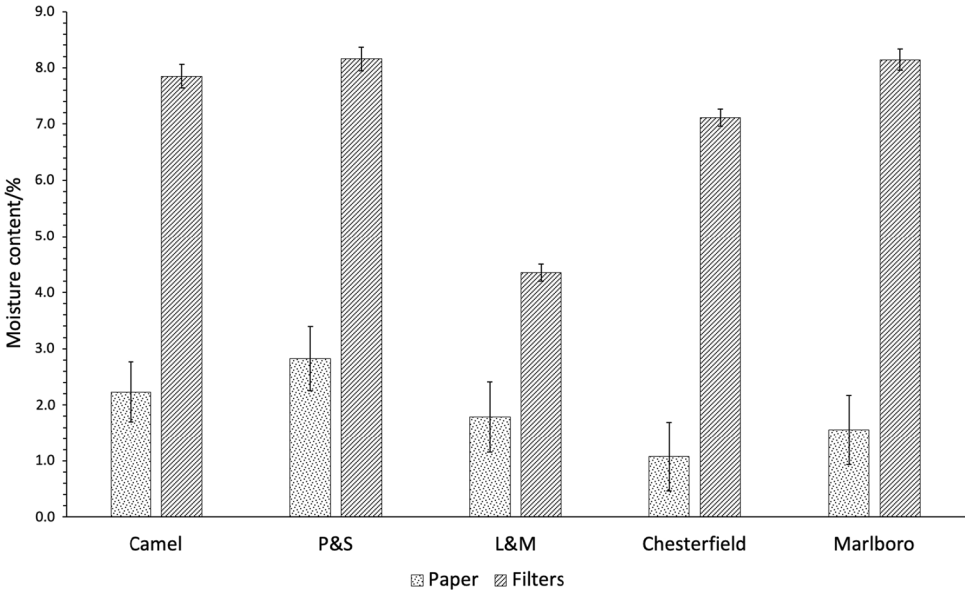

Fig. 2 Determined moisture content in five chosen cigarettes (only in filters and wrapper paper). The ranges of expanded uncertainties are also presented as an error bars for all samples

the conditions and time for conditioning tobacco samples [27]. This would imply that results should be presented in dry weight units or conditioned in accordance with the mentioned ISO requirements. The objective of this paper, however, was not to analyze the distribution of mercury from combustible tobacco, but rather to estimate consumer exposure. Therefore, the chosen approach imitates the real-world conditions under which tobacco is unpacked and consumed. The water content of the products clearly varied, as shown in Fig. 1. Determining exposure by presenting results in wet weight units, seems to more closely simulate real-world conditions than using dry weight or identically conditioned samples. It is worth noting that an analogous approach is also used to analyze food samples [24].

Despite drying, cigarette paper was so fibrous that homogenization with an agate mortar and pestle was not possible. The wrapper paper was, therefore, cut into pieces and the filters were torn, the number of repetitions of Hg analysis was increased in comparison to tobacco analysis. In future research, it would be advisable to use ball mill with ceramic or glass balls for the sample homogenization purpose.

A major advantage of the proposed approach is that the determination of the actual water content of the sample becomes eminently less important. Only the repeatable drying of the material is needed. The most important thing is to dry the samples so that homogenization is possible and to know the difference in weights before and after drying. This makes it possible to calculate the concentration in the "wet weight" of the sample. Paper and filters were treated in the same way as tobacco to keep the approach as identical as possible for all samples.

Total mercury content has been determined in four different groups of combustible tobacco products as shown in Table 1. It should be noted that only five of cigar tobacco products have been taken as representative samples for analysis of total Hg content. Total mercury concentrations range from 17 to 30 µg/kg in bidis, 25–33 µg/kg in cigarette tobacco, 16 to 24 µg/kg in pipe tobacco, and 14–27 µg/kg in cigar tobacco. Calculated uncertainties for specified values are presented in Table 1.

The magnitude of combustible tobacco consumer exposure to mercury is the result of several factors. One is the concentration of Hg in the products consumed, but equally important are consumption habits, such as the frequency of smoking. This correlates directly to the total weight of products consumed in a given unit of time. It follows that exposure is, among other things, proportional to the total weight of combustible tobacco smoked. Thus, it is important to determine what are percentages of the wrapper paper and filter in the total weight of the cigarette. Such information, together with the determined Hg content of these components, provides an estimate of whether analyzed cigarette components are likely to have a significant effect on consumer Hg exposure.

Each cigarette was divided into individual components, which were then weighed. The weight of the tobacco ranged from approximately 4.7–5.3 g, the wrapped paper weighed from 0.32 to 0.38 g, and the weight of the filters ranged from 0.96 to 1.3 g, as shown in Table 2. The obtained results correspond with those present in the literature [28].

First, the weight fraction of the analyzed materials in the cigarette should be determined. These products, however, have a certain moisture content [11]. Solid sample analysis should take this effect under consideration. It is most

Table 1 Determination of total mercury content in the wet weight of four different combustible tobacco products

Product	Brand	Mercury content/µg/kg
Cigarette tobaccos	Camel	32.89 ± 0.86
	P&S	28.4 ± 1.4
	L&M	25.41 ± 0.61
	Chesterfield	30.0 ± 2.4
	Marlboro	27.2 ± 1.1
Pipe tobaccos	Amphora Full	23.5 ± 2.5
	Peterson wild Atlantic	16.41 ± 0.43
	Mac Baren Virginia No.1	22.64 ± 1.3
	Poniatowski sungold	16.90 ± 0.87
Bidis	Rajkamal bidi	30.0 ± 1.6
	Bharath special beedies	17.7 ± 1.1
	GJ gulab bidi	28.353 ± 0.091
	Charabhai BIDI works	29.91 ± 0.57
	Sambhaji BIDI	22.84 ± 0.34
Cigar tobaccos	Oliva Serie V Lancero	13.86 ± 0.81
	A.Turrent traditional robusto natural	18.58 ± 0.20
	Te-Amo clasico magnificos	26.83 ± 0.62
	Romeo y Julieta Cedros de Luxe No.2	19.67 ± 0.62
	Principes corona caribbean	18.82 ± 0.18

Table 2 Distribution of percentage of moisture content in different elements of cigarettes calculated based on the weight of it

Cigarette brand	Weight/g of			Total weight/g	Weight Percentage/% of		
	Tobacco	Paper	Filter		Tobacco	Paper	Filter
Camel	5.26	0.38	0.96	6.59	79.70	5.71	14.59
P&S	4.90	0.35	0.96	6.21	78.88	5.65	15.47
L&M	4.78	0.32	1.33	6.43	74.37	4.96	20.67
Chesterfield	4.69	0.32	1.31	6.33	74.17	5.13	20.70
Marlboro	4.75	0.32	1.07	6.14	77.41	5.24	17.35

convenient to relate concentration in dry weight, but, as mentioned, while it is possible to compare products, it is difficult to estimate actual consumer exposure. Therefore, it is advisable to convert the concentrations to wet weight.

As can be seen, the combined weights of paper and filter are almost three times lower than the weight of tobacco. In this study only cork-tipped filtered cigarettes were analyzed; however, there are more different product features (unfiltered cigarettes, white tipped filtered cigarettes etc.) [29].

One of the filter samples stood out from the others, because the filter consisted of two components. While the first component morphologically resembled the others, the second component contained small granules of a black, hard substance in addition to fibers. These were probably pieces of carbon [30]. Importantly, there may also be differences within one product category, i.e., filtered cork-tipped cigarettes. This applies also, to the material from which the filters are made.

As shown in Fig. 3, the determined mercury content of cigarette paper and filters is lower than 11 µg/kg of wet weight. Compared to tobacco [31], the water content of the samples was significantly lower so in the end, its effect on the mercury content was not so analytically important. Nevertheless, it should always be considered during the calculation process.

In two out of five cases of cigarette brands the determined Hg content exceeded the specified LOQ. In the case of L&M, Chesterfield, and Marlboro cigarettes, the determined content of Hg was below the LOQ and sometimes even below the LOD.

The determined measurement uncertainties shown as error bars have a wide range, as presented in Fig. 3. The obtained results show low precision due to Horwitz effect (low concentration might decrease precision drastically). Therefore, an acceptable coefficient of variation factor of not more than 40% was assumed. Among the factors affecting such a low precision of the measurement are: the use of small sample weights for measurement and the influence of the Horwitz trumpet effect [32] (for low concentrations). Limit of detection (LOD) was calculated using a

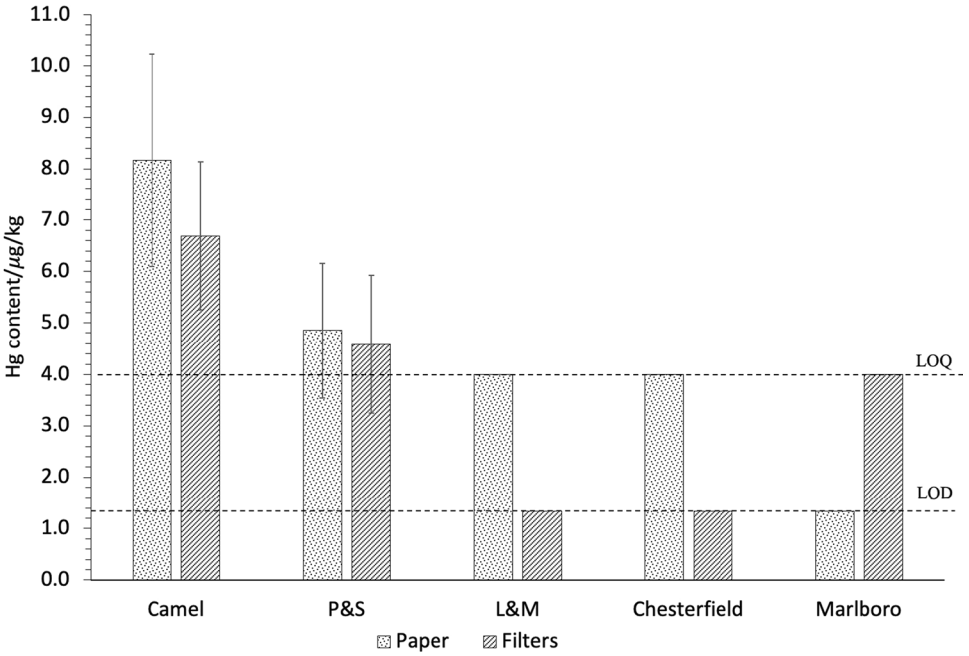

Fig. 3 Determined mercury content in wrapper paper and filters of cigarettes have been presented along LOQ = 4.0 μg/kg, and LOD = 1.3 μg/kg. The presented results are based on wet weight concentration. Mercury content in cigarette paper and filters ($C_{w.w.}$)

calibration curve. While the limit of quantification (LOQ) was calculated using the following equation:

$$\mathrm{LOQ} = 3 \bullet \mathrm{LOD} \tag{1}$$

Conclusions

The study, which employed four different types of combustible tobacco products, presented information on the high level of risk of mercury exposure following consumption of such products. Cigarettes (Camel cigarettes with 32.89 μg/kg) show a high level of total Hg content in comparison with other combustible tobacco products. The proposed research will allow to widen the knowledge and provides data allowing to evaluate the total Hg content and moisture content in the wider range of tobacco products.

It is worth remembering that one of the proven anthropogenic sources of mercury is the manufacture of paper, in which, a cigarette is eventually wrapped. In summary, this study demonstrates that it is unlikely that cigarette paper and filters could be a significant source of total human Hg exposure, particularly in comparison to tobacco. The exposure to this metal from combustible tobacco must be placed in the context of the total consumer exposure from all sources as the toxic effects might be combined (additive, synergetic or antagonictic). Relatively high Hg toxicity indicates that elimination of its every source possible is desired. It is also advisable to further test paper and cigarette filters for heavy metal content, on a wider range of samples.

Experimental

Four different kinds of combustible tobacco products were undertaken for determination of moisture content. Cigarettes and pipe tobacco used in the study were purchased from the local store (Gdansk, Poland), and represented a spectrum of popular brands chosen randomly. The cigars, on the other hand, were purchased from a Polish online tobacco shop. Bidis, not available in Poland, were imported from the random "Paan shop" (Rajkot, Gujarat, India).

All kinds of samples were dried in a laboratory dryer until a constant weight was obtained (with an accuracy of 0.001 g). Although all samples were homogenized, it was difficult to homogenize filter and wrapper paper with agate mortar or impact mill. Chosen analytical technique allows, however, solid samples analysis, so homogenization is preferred but not mandatory. Instead of homogenizing paper and filter samples its number was increased. Paper samples measurements were repeated for seven times, while filter samples measurements were repeated for eight times.

Sample preparation for analysis included sampling by cutting a slice of the product or taking tobacco from the package. Sliced pieces were then dried on watch glasses. Prior to analysis, the water-free samples were homogenized by hand with an agate mortar and pestle, and stored. Powdered samples were placed directly into the instrument's ceramic measuring boats. No additional solvents were necessary. To determine the total mercury content a cold vapor atomic absorption spectrometry technique was used. Each sample weighted approximately 100 mg in 3–4 repetitions.

Calibration of the equipment was performed prior to analysis. All calibration solutions were diluted in L-cysteine. After being weighed to 10 mg, crystalline L-cysteine (provided by Merck) was transferred to a 1000 cm^3 volumetric flask. Afterwards, to create a 0.001% L-cysteine solution, to the proper volume of stock solution, 2 cm^3 of the certified reagent grade concentrated nitric acid was added and then filled with deionized water. The reagent was stored in a cool, dark location. Hg standard of MS grade purity at a concentration of 100 mg/dm^3 was purchased from Merck. By adding 1 cm^3 of a 100 mg/dm^3 solution to a 100 cm^3 volumetric flask and diluting it to the proper concentration with the L-cysteine solution, 1 mg/dm^3 solution Hg was achieved. Subsequent solutions of concentrations 0.1 mg/dm^3 and 0.01 mg/dm^3 were prepared as described above. From these standards, the calibration curve was created by the direct introduction of proper volumes of prepared solutions into measurement ceramic boats. With such an approach, the proper correlation coefficient was achieved (0.9989). The calibration curve was obtained between the range of 1.0 to 200 ng.

The total Hg in tobacco samples was determined using cold vapour atomic absorption spectrometry. The samples, measured with at least three repetitions, were heated under controlled conditions ($T = 850$ °C/4 min) to cause thermal decomposition. A tube with gold filling (gold furnace) was used as a collections system (to create Au–Hg amalgam) to absorb and pre-concentrate free Hg vapour after further atomization of the metal. To release atomic Hg, the gold furnace was heated ($T = 600$ °C/1 min). Free Hg analysis, after described pre-concentration, was performed with the use of spectrometric analysis (wavelength 253.7 nm).

Instrumentation

Moisture content was determined by a gravimetric method using the analytical scale Radwag® AS 220.X2 (accuracy of 0.0001 g) under the atmospheric conditions: temperature 24 ± 2 °C, atmospheric pressure 104.7 ± 10 kPa and humidity 80 ± 1%. Each sample was dried using a laboratory dryer Redline By Binder under the following conditions: temperature 105 °C for 24 h supported by air circulation. Prepared samples were sorted in 50 cm^3 polyethylene Falcon® tubes protected with Parafilm®. MA-3000 mercury analyzer [Nippon Instruments Corporation (NIC, Japan)], which employed thermal decomposition, gold amalgamation, and atomic absorption (253.7 nm) to detect Hg concentration. The carrier gas was pure, dry oxygen. Standard solutions were prepared using the deionized water from Milli-Q Water Purification System (USA).

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