Original Article

Determination of Tl(I) Ions in Homeopathic Drugs by Differential Pulse Anodic Stripping Voltammetry

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ABSTRACT

Objectives: The present study is focused on the determination of thallium (I) ions in homeopathic drugs. **Methods:** The determination was carried out using the Differential Pulse Anodic Stripping Voltammetry (DPASV) technique coupled with the flow-injection measuring system (FIA-DPASV). **Results:** The thallium content was analyzed in two commercial homeopathic preparations (Thallium aceticum 5CH and 9CH), which include two different concentrations of thallium. It was established, that the mean concentration of thallium in the 5CH drug was at 6.67 ng L^{-1} in the administered daily dose, whereas in case of 9CH the value was at 2.1 ng L^{-1} of thallium. **Conclusion:** The obtained results indicate that the application of the studied homeopathic drugs for medical purposes is not associated with any hazards in terms of possible toxicity due to thallium content.

Key words: Flow-Injection Differential-Pulse Anodic Stripping Voltammetry, Heavy Metal Ions, Homeopathic Drugs, Thallium, Trace Analysis.

INTRODUCTION

Thallium is toxic to human, animal and plant organisms as well as microorganisms.[1-3] In fact, thallium exhibits higher toxicity compared to other hazardous heavy metals, such as mercury, cadmium or lead (which are characterized by a maximum admissible concentration of 0.1 mg · mL^{-1}). The threat of thallium poisoning is mainly associated with the fact that numerous thallium-based compounds display relatively high solubility in water as well as bioavailability. As a result, they are easily transported throughout the environment, enter the food chain and accumulate in living organisms.

The mechanism of toxicity of thallium is based on the similarity between thallium (I) ions and potassium ions and the resulting disorders due to disruption of enzyme activity and metabolic processes.[4-6] Upon entering the human organism, thallium rapidly spreads to numerous organs via the bloodstream and accumulates in nails, hair, bones as well as crucial organs, such as kidneys and brain. Symptoms of thallium poisoning typically include hair loss, ulcers, internal bleeding, myocardial injury, alopecia, polyneuropathy, insomnia, paralysis and loss of body mass and, eventually, death.[2,3-7] The severely negative impact of prolonged exposition to high levels of thallium is the rationale for monitoring of this contaminant in environmental samples.

The various toxic effects of thallium became a rationale for the preparation of thallium-based homeopathic drugs. Homeopathy is a set of alternative medical treatment procedures created by Samuel Hahnemann in 1796, which stem from the fundamental assumption that a substance which causes specific symptoms in a healthy person may be used to cure a sick person from similar symptoms.[8] Furthermore, the homeopathic preparations used for medical treatment are obtained as a result of serial dilutions, which supposedly facilitate the potency of the active substance. The homeopathic doctrine assumes that repeated dilution of the active substance negates its toxic effect and, at some point, allows to achieve positive effects upon

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administration.[9] However, it should be noted that mechanism of action of homeopathic drugs is not yet explained in strictly scientific terms and that homeopathic principals often directly contradict the findings of other scientific fields (i.e. biology, physics, chemistry), which contributes to a lot of controversy.[10] Moreover, the results of large-scale clinical trials indicate that homeopathy is ineffective for treatment and that the observed positive effects may be caused by a placebo effect.

Regardless, there is a growing interest in the use of homeopathic drugs, which are presently widely available. To date, homeopathy is officially considered as a medical treatment method in France and is practiced by approx. 6000 doctors. In the United Kingdom there are six homeopathic hospitals. Recently, a series of homeopathic drugs based on thallium have appeared on the market. These drugs are administered in order to treat progressing baldness since hair loss is one of the symptoms of thallium-poisoning. As a result of the growing interest in homeopathic drugs, there is a need for studies which will allow for a standardization of homeopathic drugs based on the determined content of active substances as well as their purity and the presence of potentially toxic substances, such as heavy metal ions.

The analytical methods used for determination of thallium should be sensitive and selective due to a relatively low concentration of this element as well as the presence of other compounds in chemically complex drugs. The inversion voltammetry techniques, widely used for determination of thallium in different environmental samples, exhibit a notable benefit – they are relatively cheap and the DPASV technique coupled with a flow-injection measuring system (FIA-DPASV) enables the determination of Tl at concentrations below 1 picomole per liter (of the order of 100 picograms per liter; LOD equal to 0.25 pM).[6-11,12] The aim of the present study was to determine the content of thallium in samples of the homeopathic pellets collected from a commercial brand (Thallium aceticum).

MATERIALS AND METHODS

Apparatus

The determination was conducted with the use of a MicroAutolab electrochemical analyzer from Ecochemie (Utrecht, Netherlands) coupled with a flow-through wall-jet type cell.[13] which allows for medium exchange and circulation (Figure 1). The system worked under the following conditions: differential-pulse amplitude of 50 mV, step potential of 2 mV, mercury film electrode based on glassy carbon as a working electrode, saturated calomel electrode as the reference electrode, platinum wire as the auxiliary electrode, mercury film deposition period of 10 min using a 0.05 mM mercury(II) solution of nitrate and 0.1 M potassium nitrate.

Solutions and preparations

Standard solutions of Tl (I) were prepared by dilution of a 1000 μg · mL^{-1} stock standard solution obtained from Sigma Aldrich. Ammonia solution (25%), nitric acid (65%), hydrogen peroxide (30%), EDTA and ascorbic acid (all puriss. p.a., supplied by Fluka), hydrofluoric acid (pract. 73%, Fluka) were used to conduct the determination.

All solutions were prepared in high purity water obtained by reverse osmosis in a Watek-Demiwa 5 Rosa system (Czech Republic), followed by triple distillation from a quartz apparatus. Only freshly distilled water was used.

Reference standard

In order to control the analytical quality, the precision of the proposed method during analysis of Tl in the studied samples was compared with a certified reference material - GBW 07401 soil of Chinese origin, containing 1±0.2 μg g^{-1} of Tl. Nine independent trials were conducted for the reference material in order to determine the Tl content. The average content of Tl was at 0.90±0.14 μg g^{-1} (with a minimum 0.76 of and a maximum of 1.1 μg g^{-1}). The recovery of Tl was at 90%.

Pharmaceutical preparations used in the study

The following materials, which are available at the Polish market as commercial pharmaceutical preparations, were the subject of study (Figure 2):

1. Homeopathic drug Thallium aceticum 5CH in the form of pellets (4g, 80 pellets per package) produced by BOIRON S.A. The drug has been authorized based on the license no. IL-3398/LN-H.
2. Homeopathic drug Thallium aceticum 9CH in the form of pellets (4g, 80 pellets per package) produced by BOIRON S.A. The drug has been authorized based on the license no. IL-3398/LN-H.

The abbreviation in the name of the studied homeopathic pharmaceutical preparations indicate the following: XC describes the dilution of the active substance, which can be calculated according to the following equation: $^{XC}=\frac{1}{100^{X}}$; where as H indicates that the dilution was prepared according to the Hahnemann method.

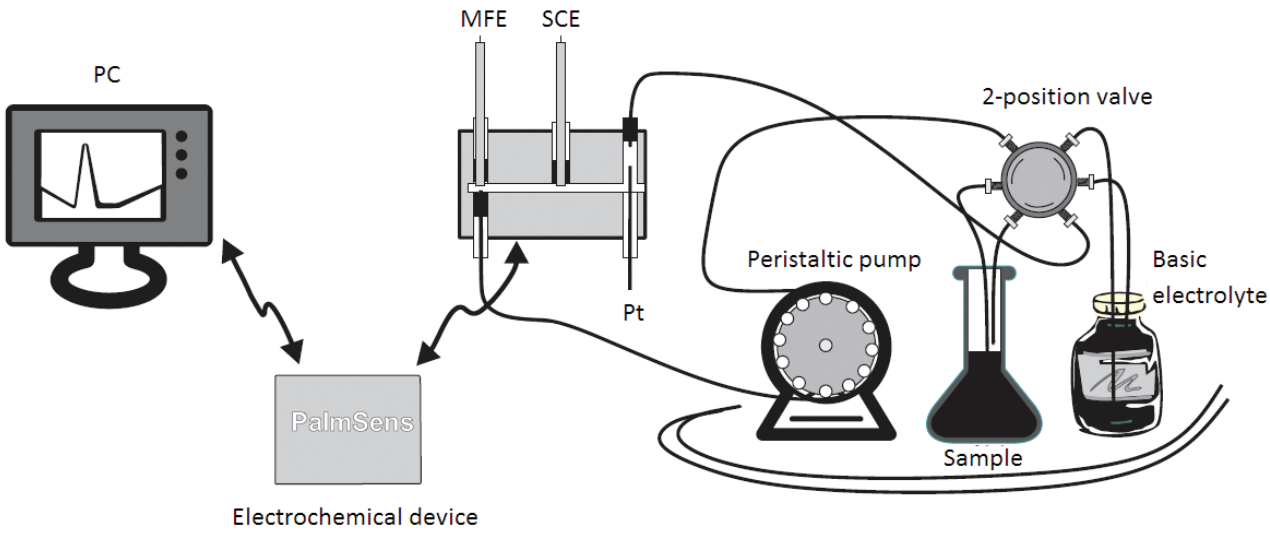

Figure 1: The Flow Injection Analysis (FIA) coupled with Differential Pulse Anodic Stripping Voltammetry (DPASV) system.

Figure 2: Commercial homeopathic drugs Thallium aceticum 9CH and 5CH.

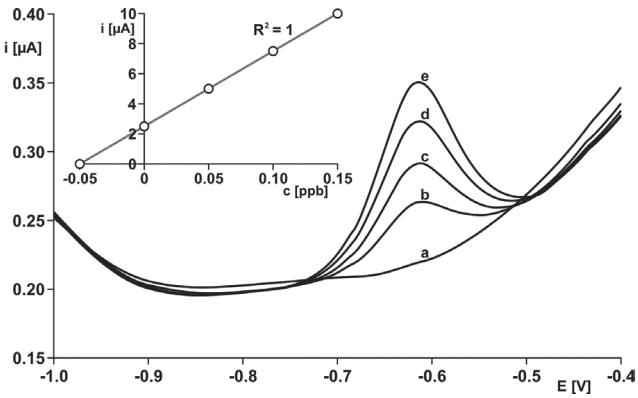

Figure 3: Representative voltammograms of thallium (for extracts of pharmaceutical preparations) in presence of 0.05M EDTA (pH 4.5) obtained with the use of a Hg/GCE electrode after 1800s of pre-concentration at -1.0 V vs. Ag/AgCl, pulse amplitude 50 mV, step potential 2 mV. (a- sample; b, c, d – sample + internal standard).

Determination of thallium in the studied pharmaceutical preparations by the DPASV

Determination of thallium in the studied pharmaceutical preparations was conducted using a previously described procedure.[13] A sample of the studied pharmaceutical preparations (0.5 g) was placed in a teflon beaker and digested by adding 65% nitric acid and 2.5 mL of 30% hydrogen peroxide. Upon evaporation of the solution the residues were mixed with an additional portion of nitric acid (1 mL), covered with a watch glass and heated for 3 hours. After filtration the residues were then mixed with ascorbic acid (2.5 mL of 1 M solution) and EDTA (6.25 mL of 0.2 M solution). The pH of the solution was then adjusted to a value of 4.5 (using an ammonium solution), then it was transferred to a flack (25 mL) and supplemented with water. This final solution was used for the determination of thallium in the studied pharmaceutical preparations using flow injection analysis differential pulse anodic stripping voltammetry (FIA-DPASV). The pre-concentration of Tl was carried out at a potential of –900 mV vs. SCE over 900–3600 seconds depending on the Tl concentration. Voltammograms were recorded after medium exchange on pure 0.05 M EDTA (Figure 3). The results were evaluated on the basis of several additions of an internal standard (typically 3 additions). The detection limit of the method (calculated on a 3SD basis) was 50 pg L^{-1} (0.25 pM).

Table 1: Results of determination of thallium (ng·L^{-1}) in the recommended daily dose (20 pellets) of the studied homeopathic drugs (n=9).

Thallium content in homeopathic drugs (daily dose 20 pellets)	Arithmetic mean [ng · L^{-1}]	SD [ng · L^{-1}]	%RSD Relative standard deviation	Median [ng · L^{-1}]	Range [ng · L^{-1}]
Thallium aceticum 5CH	6.67	0.397	5.952	6.72	6.13 - 7.22
Thallium aceticum 9CH	2.10	0.0962	4.5762	2.11	1.96 - 2.23

RESULTS AND DISCUSSION

The results of the studies have shown that the DPASV method allowed for the determination of thallium in homeopathic preparations. The results presented in Table 1 show the thallium content in the studied homeopathic drug Thallium aceticum. The concentration is very low, at trace levels (10^{-9}). Two types of dilution of the studied drug were determined – 9CH and 5CH. In accordance with the theory, the higher dilution (9CH) contains a lower concentration of Tl^{I+III}, which was confirmed by the results.

The combination of voltametric methods with a stripping technique allowed to notably decrease the detection limits and determine the total content of thallium in homeopathic drugs. In comparison, the use of Laser Excited Atomic Fluorescence Spectrometry (LEAFS) for the determination of thallium content allows to achieve a detection limit of 0.1 ng·L^{-1}.[14] Electrothermal Vaporization Inductively Coupled Plasma Mass Spectrometry (ETV-ICP-MS) allows for analysis of trace metal even at a level of 0.0009 pmol·g^{-1},[15] whereas High Resolution Inductively Coupled Plasma Mass Spectrometry (HR-ICP-MS) enables determination of thallium at a level of 0.007±0.001 nmol·L^{-1}.[16] Inductively Coupled Plasma Mass Spectrometry (ICP-MS) is characterized by a detection limit at a pg·L^{-1} level, while ETV-ICP-MS exhibits a detection limit ranging from 0.4 to 0.5 ng·L^{-1}.[17] Anodic stripping voltammetry (ASV) has long been recognized as a powerful technique for trace metals due to its sensitivity and relatively inexpensive instrumentation, and is suitable for the direct determination of trace metals without previous separation.The concentration of thallium (ng·L^{-1}) in the single dose of the studied homeopathic preparations (5 pellets administered orally) according to medical indications was presented in Figure 4. Such a low content of thallium in the studied preparations is associated with one of the main rules of homeopathy, namely principle the infinitesimal dose. Several references provide a rationale for the mechanism of homeopathy based on the so-called 'water memory' phenomenon. In theory, water has the ability to 'remember' and dynamise the medical properties during a series of dilutions and – which means that it increases its treatment capacity. These properties are supposedly stored in the vibrations of water molecules. This leads to a theory that the therapeutical effect of homeopathic drugs are not associated with chemical reactions, but instead are a consequence of the shift in their physical properties. The ranges of thallium concentrations in environmental water samples and the concentrations determined for homeopathic drugs were compared in Figure 5. The results presented in the framework of this study may be

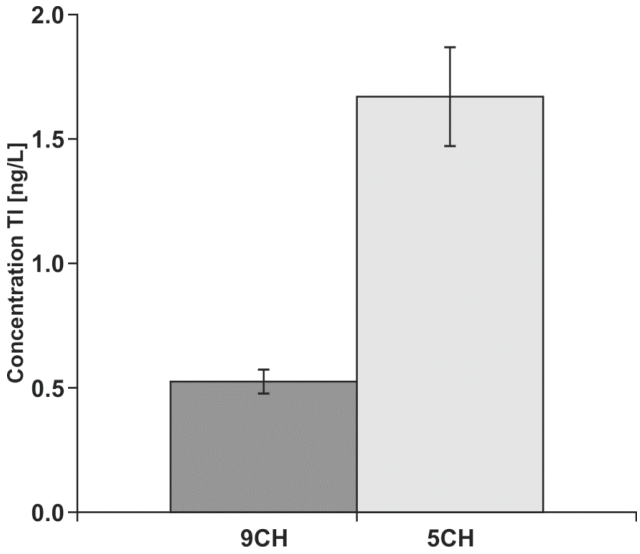

Figure 4: Mean content of thallium (ng·L^{-1}) in a single dose of the studied homeopathic preparations (5 pellets administered orally).

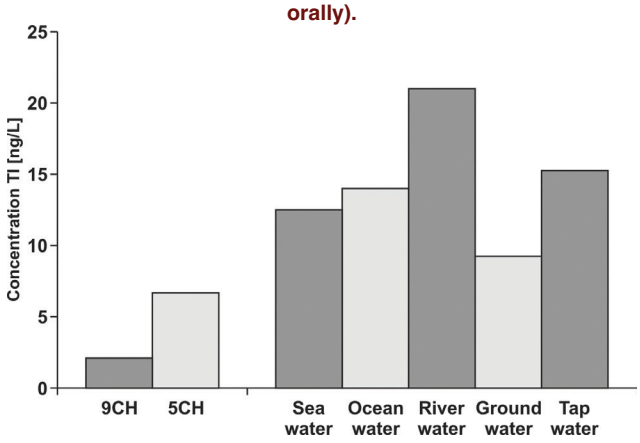

Figure 5: Comparison of the determined thallium concentrations in the studied homeopathic drugs (5CH and 9CH) administered in the recommended daily dose of the studied homeopathic preparations (20 pellets) and the content of thallium in different environmental water samples.[18,19,20]

interesting, due to the fact that the determined content of thallium in homeopathic drugs is two to ten times lower compared to environmental water samples. The content of thallium in river waters in Poland typically ranges from 5 to 17 ng L^{-1}. Sea waters usually contain 10-15 ng L^{-1} of thallium.[12-18] There is a lack of data regarding other attempts to determine the content of thallium in homeopathic drugs, therefore it was not possible to compare the obtained results with other reports.

CONCLUSION

Thallium is a highly toxic heavy metal with severe poisoning symptoms, which may ultimately result in death. Due to this reason, its content should be strictly monitored. This issue is especially crucial in case of homeopathic drugs, which are based on the administration of thallium.

The FIA-DPASV analytical method, which was employed in the framework of this study in order to determine the thallium content in the tested pharmaceutical preparations, provided satisfactory results. It can be successfully used for future determinations of trace levels of thallium in such materials

The single dose of 5 pellets of homeopathic drugs administered orally contained a notably lower amount of thallium compared to environmental water samples. Even a daily dose of such drugs contains a lower content of thallium. Based on the obtained results, it can be established that the studied pharmaceutical preparations should not be hazardous for the human health when administered according to the recommendations.

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

ABBREVIATIONS USED

FIA: Flow Injection Analysis; **DPASV:** Differential Pulse Anodic Stripping Voltammetry.

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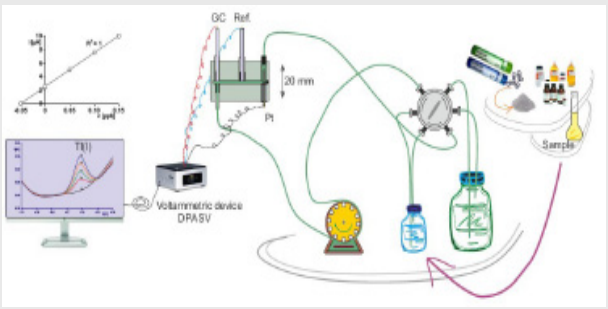
PICTORIAL ABSTRACT



SUMMARY

- The thallium content was analyzed in two commercial homeopathic preparations (Thallium aceticum 5CH and 9CH) using the Differential Pulse Anodic Stripping Voltammetry (DPASV) technique coupled with the flow-injection measuring system (FIA-DPASV). It was established, that the mean concentration of thallium in the 5CH drug was at 6.67 ng L-1 in the administered daily dose, whereas in case of 9CH the value was at 2.1 ng L-1 of thallium. The obtained results suggest that the content of thallium in the analyzed samples is lower compared to environmental samples.

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