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CASE REPORT

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Reconstruction of a case of thallium poisoning using LA-ICP-SFMS

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Abstract The unique capabilities of laser ablation in combination with inductively coupled plasma sector field mass spectrometry (LA-ICP-SFMS) were employed to reconstruct details of a homicide by thallium poisoning, which took place 38 years ago in Austria. Thallium was determined in several human bone samples after acid digestion in a microwave oven. The ICP-SFMS results showed that the thallium concentration in the victim's bones was in the range 1.07–2.63 μg g^{-1}, which is up to 170 times higher compared to concentrations found in bones of persons who have died due to natural causes. The results were in accordance with the values obtained by graphite furnace atomic absorption spectrometry (GF-AAS). Laser ablation ICP-SFMS was applied to assess the time interval between the victim's poisoning and death. Several line scans with a laser spot size of 50 μm were performed on a thumbnail of the poisoned person and on a reference thumbnail by laser ablation ICP-SFMS. Thallium peaks were detected on the nail of the victim at a distance of 2.5 mm from the younger edge of the nail.

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Introduction

In recent years the use of thallium (Tl) has decreased due to its toxicological and environmental impact. Today Tl is mainly utilized as a catalyst for organic syntheses, in electrical and electronic industries and in the production of special glasses. Thallium is released into the environment mainly from mineral smelters, coal-burning power plants, brickworks and cement plants (Fischer and Eikmann 2002). Hence, the metal is detected in all environmental compartments. Foodstuff contains <1–3 μg kg^{-1} Tl, the average dietary intake of Tl amounts to 2–4 μg. (Ysart et al. 1999). The inhaled amount of Tl has been estimated to <0.005 μg per day (WHO 1996). Physiological background concentrations of Tl in the range of 5 μg l^{-1} in urine, 2 μg l^{-1} in blood and 20 μg kg^{-1} in scalp hair have been recorded (Bertram and Kemper 1983).

Some decades ago, Tl containing compounds were applied for treatment of infectious diseases (e.g. syphilis, gonorrhoea) and as a depilatory. Furthermore, thallium sulfate (Tl_2SO_4) was the major ingredient of freely available rodenticides, leading to frequent abuse as a homicide agent. Sub-acute lethal intoxications provide a typical clinical pattern with neurological symptoms and a characteristic loss of hair, especially at the outer parts of eye brows. The reduced availability of Tl in everyday life has led to the fact that the element is only in rare cases considered as the cause of an observed neurological disease or in connection with its lethal consequences. Even in cases of high autopsy numbers, forensic pathologists are nowadays rarely faced with the analysis of a case of Tl intoxication.

Similar to other heavy metals and some organic compounds, thallium is deposited in human organs and tissues. Kidney, heart, liver, bones and cartilage as well as hair and nails are preferred depots for the metal (Fischer and Eikmann 2002). The excellent suitability of post-

mortem bone samples for forensic investigations has been demonstrated in several studies in recent years. Analysis of trace element concentrations and isotopic composition of bone material has been exploited for determination of the post-mortem interval (Swift 1998; Neis et al. 1999; Swift et al. 2001). Furthermore, skeletal remains have been recognized as a highly valuable material for confirming cases of poisoning (Kudo et al. 1997; Raikos et al. 2001; De Wolff et al. 2002; Karger et al. 2004). In this context, bone samples are expected to represent an ideal object for the forensic investigation of cases of thallium poisoning.

Several analytical methods are available for direct elemental analysis of Tl in biological and medical samples (Rios and Galvan-Arzate 1998). Atomic absorption spectrometry and atomic emission spectrometry (Aziz et al. 1982; Hee and Boyle 1988) as well as inductively coupled plasma mass spectrometry (ICP-MS) (Nixon and Moyer 1996; Mestek et al. 2000), have been applied for quantification of Tl at elevated and background concentrations. For direct determination of the longitudinal distribution of Tl in human scalp hair, 10 mm hair segments have been analysed by ICP-MS (Maurice et al. 2002). However, methods using conventional sampling strategies (i.e. cutting hair segments) are limited in lateral resolution and are therefore not capable of delivering continuous data on the time-dependent course of Tl administration and its consequent deposition in hair and nail samples.

In this situation laser ablation inductively coupled plasma sector field mass spectrometry (LA-ICP-SFMS) offers excellent spatial resolution (20 µm) in combination with ultimate sensitivity and spectral resolution. In the present study, the method has been applied to obtain a signal versus time profile of Tl in the thumbnail of a person buried for 29 years. Since nails reflect the health events of the previous months, one aim of the study was to investigate if a reconstruction of thallium poisoning is feasible by application of LA-ICP-SFMS. Moreover, solution-based ICP-MS has been employed for determination of Tl concentrations in the bones of the victim after wet microwave digestion of the sample material.

Case report

During a divorce suit in 1993 the husband accused his own wife of having poisoned his father 27 years ago. Consequently, the judge ordered the police to make inquiries about the circumstances in connection with his death. During these investigations, the 72-year-old mother of the suspected woman gave evidence that at the burial in 1966, her daughter confessed to having poisoned her father-in-law with a rodenticide. According to her daughter's description the victim developed a neurological disease including paralysis of the lower limbs. Subsequently, he lost hair and several nails and became a nursing case. After approximately 3 weeks the daughter-in-law decided to suffocate him by putting a plastic bag over his face. However, the mother of the suspected woman kept the confession to herself until 1993 and the death was assigned to apoplexy (stroke). A post-mortem examination had never been performed.

However, in the investigations of the police in 1993, the accused woman contradicted her mothers statements. Consequently, the remains of the victim were exhumed. During the examination of the skeleton by forensic experts, one thumbnail was recovered. Additionally, several bone samples (thigh bone, parietal bone, skull) and putrefied material were collected as evidence for further analytical investigations. Graphite furnace atomic absorption spectrometry (GF-AAS) of the samples revealed elevated Tl concentrations, which caused the court to accept the mother's statements leading to the conviction of the accused woman for having killed her father-in-law.

However, the analytical method applied in this case provided only total Tl concentrations, which could not give any information about the interval between Tl application and time of death. Furthermore, it could not prove if either Tl poisoning or suffocation has been the cause of death. Therefore, the suitability of LA-ICP-MS was tested for obtaining transient (time-dependent) Tl signal profiles from human nails.

Materials and methods

Chemicals and standards

The sample preparation procedure for GF-AAS comprised ultrapure distilled water and analytical reagent grade nitric acid (Merck, Darmstadt, Germany). A 1000 mg l^{-1} Tl single element standard (Merck, Darmstadt, Germany) was employed for quantification of Tl.

For ICP-SFMS measurements all chemical preparations were conducted on special class 100 workbenches located within a class 100'000 clean laboratory. Ultrapure water was obtained using a reagent I grade water (>10 MOhm cm resistance according to ISO 3696 water specifications) purification system (HQ, USF, Vienna, Austria) and was further purified in a quartz sub-boiling system (Milestone-MLS, Leutkirch, Germany). Analytical reagent grade nitric acid (VWR, Darmstadt, Germany) was additionally cleaned by double sub-boiling distillation in an ultrapure quartz apparatus (Milestone-MLS, Leutkirch, Germany). Ultrapure H_2O_2 (30%) was purchased from Fluka (Buchs, Switzerland).

In addition 1000 mg l^{-1} single element ICP-MS standard solutions (VWR, Darmstadt, Germany) were used throughout the study to prepare standard solutions.

Sampling and sample pre-treatment

During the exhumation, several bone samples and putrefied material of the victim were recovered. The nail sample was found connected to the hand bone. As a reference, bone samples were taken of a male person who had been buried in the same grave in 1925. The person had died at the age of 60 years due to natural causes. The reference bones were situated above the victim's samples excluding any Tl contamination

Prior to sample digestion for GF-AAS, the bone samples were pre-cleaned in 10% nitric acid and rinsed with ultrapure distilled water.

For ICP-SFMS, representative pieces of bone samples were cut from one thigh bone and one parietal bone of the skull using a

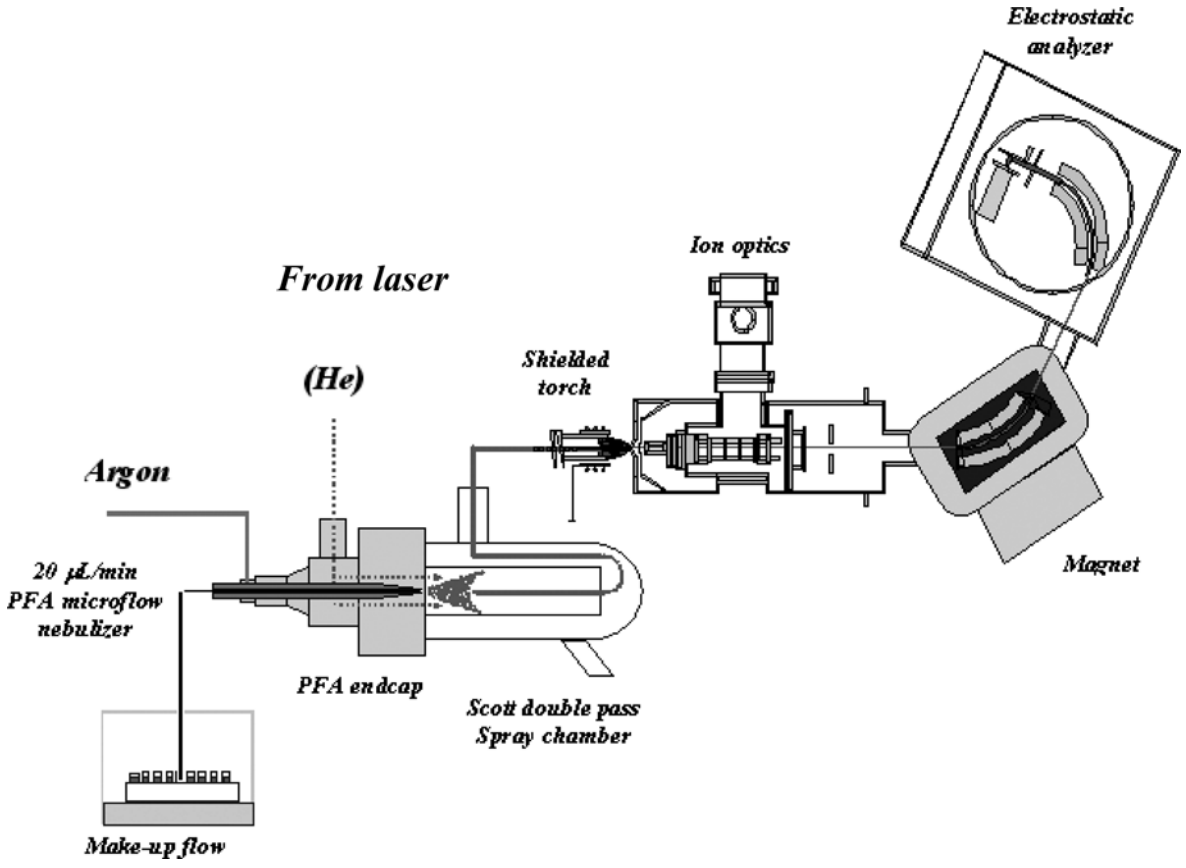

Fig. 1 Set-up of the laser ablation inductively coupled plasma mass spectrometry (LA-ICP-SFMS) system

diamond saw. The pieces were pre-cleaned in 1% HNO_3 in an ultrasonic device, dried at 105°C, mechanically cleaned in a bath of 65% HNO_3, rinsed with sub-boiled H_2O and dried again at 105°C. The dry samples were milled and homogenized in a Teflon ball-mill.

The nail sample was pre-cleaned using 1% HNO_3followed by ultrapure water.

Acid digestion

Sample decomposition for GF-AAS was achieved via high pressure digestion (High pressure asher, Anton Paar, Ostfildern, Germany) using 2 ml of concentrated nitric acid. Prior to measurement all sample solutions were brought to 10 ml using ultrapure distilled water.

For ICP-SFMS measurement, acid digestion of the bone material was performed in a microwave oven (MLS mega 1200, MLS, Leutkirch, Germany) using 150 mg of sample, 3 ml HNO_3 and 1 ml H_2O_2. Following the power program listed in Table 1, the samples were decomposed at a maximum temperature of 180°C and a pressure of 2.5 MPa. After cooling, the samples were brought to 20 g using ultra-clean sub-boiled water.

Table 1 Microwave digestion procedure

Step	Time (min)	Power (watt)
1	00:01:00	250
2	00:02:00	0
3	00:20:00	250
4	00:06:00	600
5	00:05:00	650

GF-AAS measurements

A Perkin Elmer 5100 AA atomic absorption spectrometer was employed for the assessment of Tl concentrations in bone samples and putrefied material. A magnesium/palladium modifier was used to reduce background and interference. Thallium quantification was performed via standard addition.

ICP-SFMS measurements

Thallium was determined by ICP-SFMS (ThermoFinnigan MAT Element1, Bremen, Germany). The liquid samples were introduced by a microconcentric nebulizer (MCN100, Cetac Technologies, Omaha, NE) in combination with a "Scott-type" double pass spray chamber, which was cooled at 4°C. The sample up-take of approx. 80 µl min^{-1} was achieved using a peristaltic pump (tube internal diameter 0.25 mm). Thallium was quantified within a range of 0.01–0.2 ng g^{-1} via external calibration using indium (1 ng g^{-1}) as internal standard. The calibration curve showed a linear relationship with a correlation coefficient (R^2) of 0.9998.

LA-ICP-SFMS measurements

The set-up of the LA-ICP-SFMS coupling is shown in Fig. 1. A Nd:YAG 266 nm laser system (LUV266X, New Wave Research, Fremont, CA) was coupled to a ICP-SFMS (Finnigan MAT Element2, Bremen, Germany). The isotopes ^{203}Tl, ^{205}Tl and ^{32}S were monitored at medium mass resolution ($m/\Delta m$=4500) using sulfur as internal reference element. The ablated material was transported by a 0.7 ml min^{-1} He gas flow towards the ICP, where the ablated particles were moistened in a "Scott-type" spray chamber

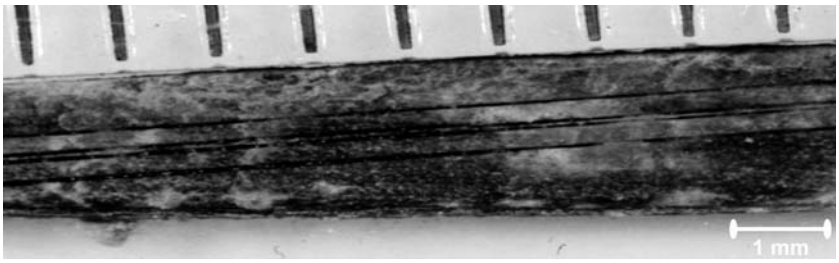

Fig. 2 Cut strip of the victim's nail showing 4 line scans performed with the 266 nm laser beam with a spot size of 50 µm. The scans were made at the inner side of the pre-cleaned nail

by adding a wet aerosol through the nebulizer before entering the plasma. Several line scans (spot size 50 µm, scan rate of 10 µm s^{-1}) were performed with laser energy of 0.7 mJ and a repetition rate of 4 Hz at the inner side of the pre-cleaned thumbnail.

Results and discussion

Thallium in bone samples and putrefied material

The GF-AAS measurements carried out in 1995 revealed elevated Tl concentrations in all investigated samples. The bone samples contained 2 µg g^{-1} (skull), 2 µg g^{-1} (rib) and 4 µg g^{-1} Tl (thigh). The putrefied remains of liver and kidney and the nail sample contained 7, 11 and 5 µg g^{-1} Tl, respectively. Unfortunately no reference values on the background concentration of Tl in bones were available. In order to get information about natural background concentrations in bone material, two reference bones (rib, thigh) of a person who died due to natural causes were analysed for their corresponding thallium concentration. In both cases the Tl concentration was below the methods limit of detection (0.05 µg g^{-1}). These values and the background values determined by ICP-SFMS are in the range of the Tl levels of <0.01 µg g^{-1} in human tissue published by Mulkey and Oehme (1993).

In our study the suitability of ICP-SFMS as a tool for ultratrace analysis of Tl in bone samples was evaluated. Two different bones types (i.e. skull and thigh) were investigated for their content of Tl. As it can be seen in Table 2, as in the case of the GF-AAS measurements, the bone samples of the victim provided elevated Tl concentrations, indicating evidence for Tl poisoning. The Tl concentrations of the investigated bone samples are in good agreement with the GF-AAS results.

Table 2 Thallium concentrations in bones of the poisoned person and in reference bones of a person who died due to natural causes

Bone samples	Victim AAS µg g^{-1}	Victim ICP-SFMS µg g^{-1}	Reference ICP-SFMS µg g^{-1}
Skull	2	2.63	0.094
Thigh	4	1.76	0.010
Thigh (head)	-	1.07	0.054

The procedural limits of detection of the GF-AAS and ICP-SFMS methods are 0.05 and 0.001 µg g^{-1}, respectively.
The expanded uncertainty of the ICP-MS and GF-AAS values are 8 and 15%, respectively.

Thallium analysis in thumbnail samples by LA-ICP-SFMS

To study the time-dependent Tl administration to the victim, the transient thallium concentration of the recovered thumbnail sample was assessed by LA-ICP-SFMS. Figure 2 shows a part of the thumbnail analyzed by four line scans (dark lines) using the 266 nm laser beam with a spot size of 50 µm and a scan rate of 10 µm s^{-1}. During scans each 10 mm long, corresponding to an analysis time of 16.67 min, the signals of ^{203}Tl, ^{205}Tl and ^{32}S were acquired by ICP-SFMS using a fixed mass resolution of 4500 ($m/\Delta m$ assessed at 5% peak height). Measurement at this mass resolution is a prerequisite since $^{32}S^{+}$ is overlapped by $^{16}O_2^{+}$ (spectral interference) at a mass resolution lower than 1800 (Prohaska et al. 1999). Sulfur was chosen as an internal reference element due to the fact that it is representatively distributed in the nail (sulfur-containing cysteine is a major compound of skleroprotein keratine), therefore, independent of possible heterogeneity caused by post-mortem degradation. The signals, which were obtained by LA-ICP-SFMS are shown in Fig. 3. The peaks at 2.5 mm distance from the youngest edge of the nail gave evidence for the ingestion of a high amount of Tl. The fact that Tl was only observed in the inner pair of line scans (see Fig. 2) suggests that the horizontal integration of the element into the root of nail is inhomogeneous.

According to the literature, high-dose Tl exposures cause so-called lunular or Mee's stripes in the nails as the result of impaired growth (Prick 1979). In the present case, Mee's stripes did not occur and no disturbance of nail growth could be observed. These facts and the data obtained from the liquid analysis of the thallium concentration in the bone samples indicate a low-dose intoxication, which is in accordance to the medical history of the case. Evidently, the blue warning colour of the rodenticide-paste prevented the administration of high amounts of poison via food or drinks (the paste used contained approximately 2–3% Tl_2SO_4, the minimal lethal dose of Tl is 8 mg kg^{-1} body weight (Mulkey and Oehme 1993).

The exact lateral location of the Tl signals obtained for the two medium line scans was employed to estimate the time interval between Tl administration and time of death. Assuming a nail growth rate of approximately 0.5 and 1.2 mm per week (Beaven and Brooks 1984), it can be concluded that the man was poisoned 2–5 weeks prior to death and that intoxication did not imperatively cause the death of the person. This is in agreement with the

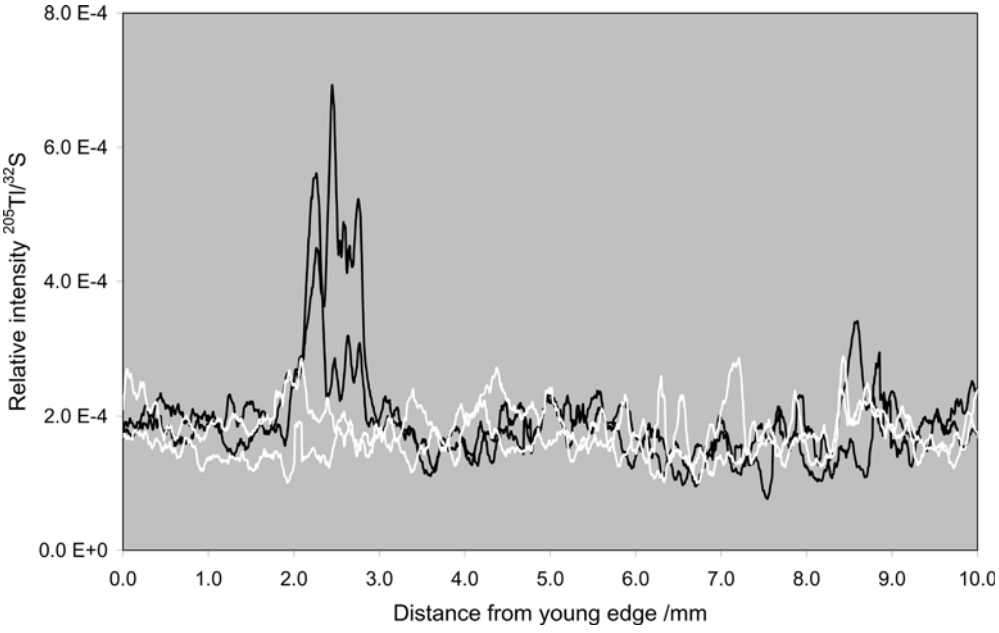

Fig. 3 LA-ICP-SFMS on the victims nail corresponding to the line scans in Fig. 2. The white and black lines represent the signals obtained for the two outer and inner line scans, respectively. In order to correct for signal drift and sample heterogeneity, the ^{205}Tl signal was normalized to ^{32}S

statement of the offender's mother, to whom she confessed to having suffocated her father-in-law with a plastic bag 4 weeks after the attempt to poison him with a thallium-containing rodenticide.

Conclusions

The applicability of LA-ICP-SFMS for generation of time resolved data on human nails has clearly demonstrated the potential of this method for tracing trace element administration. Future studies employing LA-ICP-MS in combination with isotope dilution analysis will aim at the accurate quantification of Tl in nail and hair samples.

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