Toxichem Krimtech 2011;78(Special Issue):262

Systematic toxicological analysis revealing a rare case of captan ingestion

Anne-Kathrin Gottzein, Frank Musshoff, Christian Schyma, Burkhard Madea

Universitätsklinikum Bonn, Institut für Rechtsmedizin, Stiftsplatz 12, D-53111 Bonn, Germany

Key words: Captan, systematic toxicological analysis, GC-MS, solid-phase microextraction, suicide, tetrahydrophthalimide

Abstract

Aim: A case of suicide by intoxication with various pharmaceuticals, especially anticonvulsants, combined with the fungicide captan is presented. Based on the autopsy no cause of death was ascertained; a systematic toxicological analysis, which also included a screening via solid-phase microextraction (SPME) and gas chromatography-mass spectrometry (GC-MS) for (semi)volatile organic compounds, generated important evidence.
The significance of a complete systematic toxicological analysis should be emphasised. The effect of captan on the human organism, its metabolism and distribution will be discussed.

Methods: The screening is carried out by means of SPME and GC-MS. The determination and quantification of the captan metabolite tetrahydrophthalimide (THPI) take place by liquid-liquid extraction and GC-MS analysis.

Results and Discussion: Due to the fact that captan degrades by contact with thiols, it was found exclusively in gastric contents. For the first time, THPI was detected in human viscera; the distribution in the organism corresponds to the findings in rats. The total amount of THPI in gastric contents was 1.5 mg; concentrations decreased in the following order: heart blood (0.35 µg/ml), bile (0.30 µg/ml), liver (0.24 µg/ml), femoral blood (0.22 µg/ml), kidney (0.14 µg/ml) and cerebrum (0.06 µg/ml).

Conclusion: The metabolite THPI would have been missed without previous determination of captan. From a scientific point of view, a rare case like this captan ingestion would have been disguised. And last but not least this fatality could not be investigated satisfactorily.

1. Introduction

1.1. Case report

The body of a 40-year old woman was found in her bathroom lying in a supine position. Some indefinable adherences and contents were found at the toilet. There was a noticeable amount of empty and partially used packages of tablets, respectively. Acetazolamide, levetiracetam, lacosamide, agomelatin, lamotrigine, citalopram, clobazam, and lorazepam belong to these one found which are only available on prescription. The door of the flat was open and the telephone receiver was picked up.

She suffered from intense epilepsy with mainly complex focal seizures. Because of her disease her family kept close contact. Therefore, she was found by her brother-in-law at 8.30 p.m. (last time seen was 4 p.m.) after she was not available by phone.
A day before her death she had an appointment and hoped very hard to get back her driver's license. However, the doctor told her that this is not possible as long as she is not free of seizures for minimum one year, rather two. Furthermore, there was found an empty envelope with the handwritten request to take care about her pets and order a dumpster.

1.2. Autopsy findings

The cause of death was macroscopically non-specific. A rawboned body with 47 kg body weight (height 171 cm) and gray-bluish gastric contents with a pungent smell were conspicuous. In addition, she had a vagus nerve stimulator (decommissioned) and was in status post trepanation.
Also the histological findings were unspecific. The gastric mucosa was very well preserved and showed no inflammation; the heart muscle was microscopically without any abnormalities as well.

1.3. Systematic toxicological analysis

A systematic toxicological analysis was followed up. Table 1 shows an overview of the performed analytical methods.

Tab. 1. Overview of performed analytical methods.

Method	Mode	Matrix
Immunochemical tests (ELISA)		Serum, urine
Liquid chromatography with diode array detector (HPLC-DAD)	Quantification	Serum, Femoral blood
Liquid chromatography-tandem mass spectrometry (LC-MS/MS)	Screening	Urine, gastric contents
	Quantification	Femoral blood
Solid-phase microextraction with gas chromatography-mass spectrometry (SPME-GC/MS)	Screening	Femoral blood, gastric contents
Gas chromatography-mass spectrometry (GC-MS)	Screening	Heart blood, gastric contents, kidney
	Quantification	Heart blood, femoral blood, gastric contents, kidney, bile, liver, cerebrum

1.4. Captan

Captan is a broad-spectrum agriculture fungicide that has been in use for over 60 years. It is deployed mainly to control disease in vegetables and fruits, but also as an additive in paints to reduce colonization by algae and growth of seaweeds in boats [1;2].

In fungicides, captan inhibits the endogenous respiration, the citrate synthesis from acetate, and the carbohydrate and amino acid dissimilation. Phosphate accumulates in poisoned spores, whereas lipid, protein, and nucleic acid decrease. Furthermore, there is an inhibitory action on glycolysis [3].

Captan is very labile in the human organism. It is decomposed by thiols with scission on the N-S-bond. The biotransformation in mammalian species generates tetrahydrophthalimide (THPI) and thiophosgene. Latter is very reactive; conjugation with glutathione leads to 2-thiothiazolidine-4-carboxylic acid (TTCA) [4].

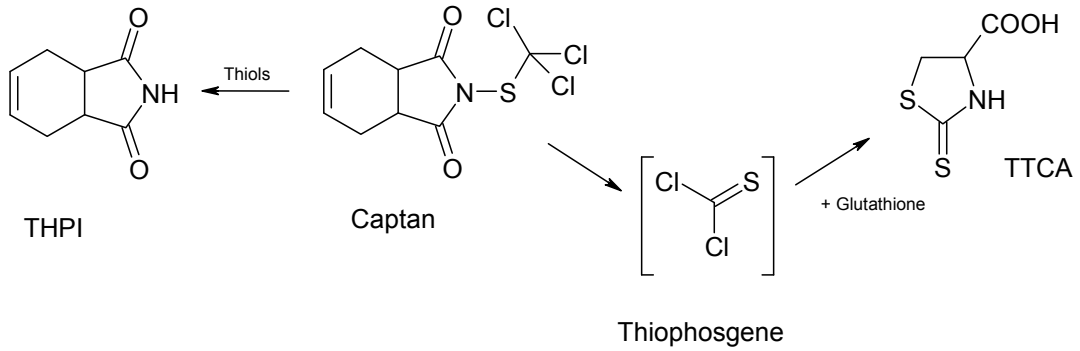

Fig. 1. Metabolism of Captan.

2. Material and Methods

2.1. Materials

All solvents used were of analytical grade. Acetone, petrol ether, sodium chloride and water (HPLC-grade) were obtained from E. Merck (Darmstadt, Germany). THPI was purchased from Sigma-Aldrich (Steinheim, Germany).
Syringe and a SPME fiber assembly [100 µm polydimethyl siloxane (PDMS) coating] were obtained from Supelco (Bad Homburg, Germany).

2.2. Instrumentation

GC-MS analysis was performed using a HP Model 5890 Series II Plus (Hewlett-Packard, Waldbronn, Germany) with an HP 5972 Mass Selective Detector. Data acquisition and analysis were performed using standard software supplied by the manufacturer. Peak identification was performed via Pfleger-Maurer-Weber [5] library. The system was equipped with a 30 m × 0.25 mm i.d. fused silica capillary column (Hewlett-Packard, HP-5-MS, 0.25-µm film thickness); carrier gas: helium (pressure 40.1 kPa); injector temperature: 260°C; transfer line temperature: 280°C.

2.3. Sample preparation for SPME-GC/MS

Because of the bluish coloration of the gastric contents and the pungent smell an analysis for volatile organic substances was performed.
In a 10 ml headspace vial, an aliquot of the sample (1 ml blood and gastric contents, respectively) was diluted with 2 ml water and the vial was closed quickly. The sample was incubated for 90 s at 40°C. Then, the fiber was exposed to the vaporous phase for 20 min, the temperature was held at 40°C and the vial was agitated during the extraction process. Afterward, desorption takes place for 3 min at 260°C in the GC inlet. A liner with a small diameter especially for SPME purpose was used [6].
Temperature program: 36°C for 4min, 10°C/ min to 120°C, 20°C/ min to 280°C, hold 3 min (total run time 24.40 min)

2.4. Sample preparation for the quantification of THPI via GC-MS

For the quantification of THPI a GC-MS method by Angioni et al. [7] was modified to perform analysis of human viscera instead of grapes.

A 1 g aliquot of (homogenized) sample (femoral blood, heart blood, gastric contents, bile, liver, kidney, and cerebrum) was weighed in a screw-capped vial; 0.8 g NaCl and 2 ml acetone/ petroleum ether (50:50 v/v) were added, and the vial was agitated in a rotary stirrer for 15 min. The phases were allowed to separate; the organic phase was vaporized to dryness and reconstituted in 100 µl ethyl acetate.
Temperature program: 80°C for 1 min, 12°C/ min to 280°C, hold 1 min (solvent delay 4 min, total run time 18.67 min)
The analysis was performed in selected ion mode: 51, 79, 106, 122, 151 (quantifier ion is underlined).

3. Results and Discussion

Via SPME-GC/MS for volatile organic compounds captan could be detected in gastric contents. Of course, it could not be determined in femoral blood because of the thiols and the associated degradation described above.

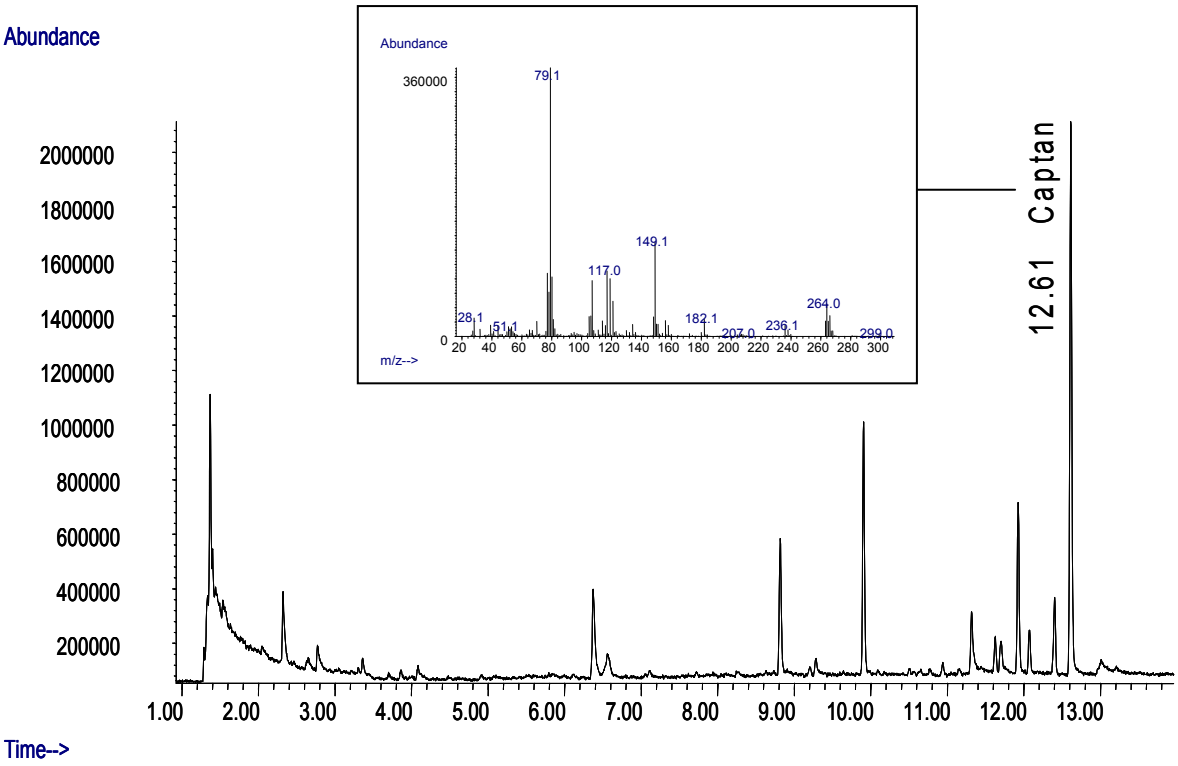

Fig. 2. Total ion chromatogram (TIC) of gastric contents (SPME-GC/MS).

Initially, the screening via GC-MS seemed to do not achieve any result. The TIC showed an enormous peak that was declared as ‘impurity’ by the library. Afterward, with the knowledge that the victim had ingested captan the peak could related to THPI.

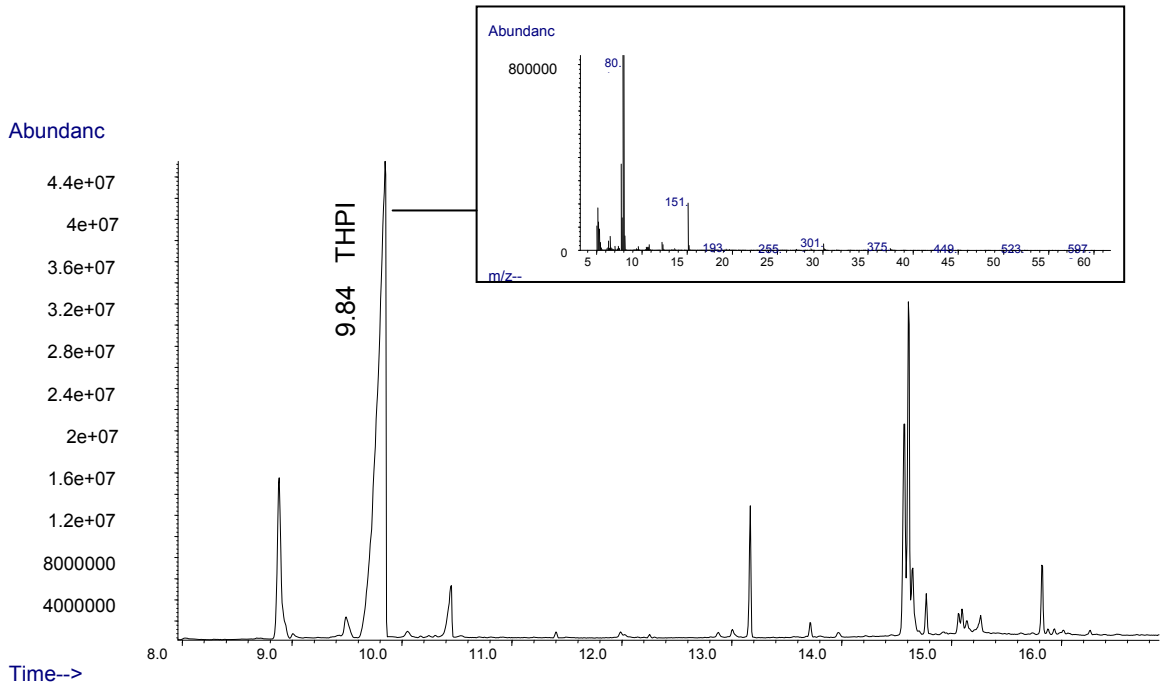

Fig. 3. TIC of gastric contents (screening GC-MS).

All results of the systematic toxicological analysis are summarized in Tab. 2. As it is shown THPI was detected in all analyzed matrices.

Tab. 2. Results of the systematic toxicological analysis.

Method	Matrix	Analyte
ELISA	Urine	Benzodiazepines
HPLC-DAD	Femoral blood	Clobazam (50 ng/ml), citalopram (469 µg/ml), citalopram-metabolite (172 µg/ml), lamotrigine (2310 ng/ml)
LC-MS/MS	Femoral blood	Levetiracetam (26.69 µg/ml), lacosamide (11.3 µg/ml)
	Gastric contents	Clonazepam
SPME-GC/MS	Gastric contents	Captan
GC-MS	Diverse	THPI (gastric contents about 1.5 mg, heart blood 0.35 µg/ml, bile 0.30 µg/ml, liver 0.24 µg/ml, femoral blood 0.22 µg/ml, kidney 0.14 µg/ml, and cerebrum 0.06 µg/ml

For the first time, THPI was detected in human viscera. The distribution of THPI in the organism corresponds to the findings by Seidler et al. [8]. This work group carried out some experiments on rats in 1971.

The LD_{50} of Captan is 9 g/kg; THPI for itself is not toxic. Nevertheless, the fatality is given due to diverse aspects. First, the body weight of the victim amounted to 47 kg with a height of 171 cm. The LD_{50} decreases significantly at low-protein diet or starvation [9]. Second, lacosamid is slightly overdosed and the citalopram concentration is above the therapeutic range. Latter is not sufficient to explain fatality [10]; the combination of the fungicide captan and the partially overdosed pharmaceuticals have to be considered as the cause of death.

4. Conclusion

To the exclusion of competitive causes of deaths the combination of captan, citalopram, and lacosamid in high concentrations turned out to be fatal. Cardiotoxic effects especially caused by captan are assumed.
In this case, gastric contents have shown to be of great importance as screening matrices. Without its SPME analysis for volatile organic compounds, captan and its metabolite THPI would not have been detected. In this respect, the death had not been resolved. The systematic toxicological analysis must always be adapted to the facts to produce satisfying and true results.

5. References

[1] Boran H, Capkin E, Altinok I, Terzi E. Assessment of acute toxicity and histopathology of the fungicide captan in rainbow trout. Exp Toxicol Pathol 2010;in press.

[2] Lambropoulou DA, Konstantinou IK, Albanis TA. Determination of fungicides in natural waters using solid-phase microextraction and gas chromatrography coupled with electron-capture and mass spectrometric detection. J Chromatogr A 2000;893:143-156.

[3] Gale GR, Smith AB, Atkins LM, Walker EM, Gadsden RH. Pharmacology of Captan: Biochemical Effects with Special Reference to Macromolecular Synthesis. Toxicol Appl Pharmacol 1971;18:426-441.

[4] Arce GT, Gordon EB, Cohen SM, Singh P. Genetic toxicology of folpet and captan. Crit Rev Toxicol 2010;40:546-574.

[5] Maurer HH, Pfleger K, Weber AA. Mass spectral and GC data of drugs, poisons, pesticides, pollutants and their metabolites. Wiley-VCH, Weinheim, 2007

[6] Gottzein AK, Musshoff F, Madea B. Qualitative Screening for volatile organic compounds in human blood using solid-phase microextraction and gas chromatography-mass spectrometry. J Mass Spectrom 2010;45:391-397.

[7] Angioni A, Garau VL, Aguilera del Real A, Melis M, Minelli EV, Tuberoso C, Cabras P. GC-ITMS Determination and degradation of captan during winemaking. J Agric Food Chem 2003;51:6761-6766.

[8] Seidler H, Härtig H, Schnaak W, Engst R. Untersuchungen über den Metabolismus einiger Insektizide und Fungizide in der Ratte. Die Nahrung 1971;15:177-185.

[9] Engst R, Raab M. Zum Metabolismus fungizider Phthalimid-Derivate in lebensmittelchemisch-toxikologischer Sicht. Die Nahrung 1973;17:731-738.

[10] Anastos N, McIntyre IM, Lynch MJ, Drummer OH. Postmortem concentrations of citalopram. J Forensic Sci 2002;47:882-884.