1 Summary

A method validation according to guideline SANCO/825/00, rev. 7 (17/03/2004) for the determination of captan in drinking water and surface water was successfully performed.

Captan was determined using following procedure:

Extraction:	Solid-phase extraction using polymer styrene divinylbenzene
Elution solvent:	Acetone
Final analysis:	GC/MS
Limit of quantification:	0.1 µg/L
Limit of detection:	0.03 µg/L (30 % of LOQ)
Blanks:	In untreated control samples for the current method validation captan was not detectable.
Fortification levels:	0.1 µg/L, 1 µg/L
Calibration range:	0.03 µg/L to 1.5 µg/L

2 Dates

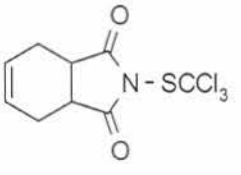
Study initiation date:	07 February 2005
Start of the experimental phase:	03 March 2005
End of the experimental phase:	15 March 2005
Draft Report:	24 March 2005
Study completion date:	27 March 2005

3 Study Objective

The aim of the current study was to validate an analytical method according to guideline SANCO/825/00 for determination of residues of captan in drinking water and surface water.

4 Materials and Methods

4.1 Test Substance

Common name:	Captan
Structure:	
Molecular formula:	$C_9H_8Cl_3NO_2S$
Molecular weight:	300.6
CAS number.:	133-06-2
Supplier:	Sigma-Aldrich, Seelze/Germany
GAB code:	20051076
Batch number:	4068x
Appearance:	crystalline colourless solid
Purity:	99.1 %
Certificate of analysis:	05 April 2004
Expiry:	08 March 2011
Storage conditions:	refrigerated (4 – 10 °C nominally)

The test substance was also used as reference substance.

4.2 Preparation of Standard Solutions

From the test substance a stock solution containing 1000 µg/mL in acetone was prepared. Three dilutions containing 100 µg/mL, 10 µg/mL and 1 µg/mL were prepared in acetone.

All standard solutions were stored refrigerated (nominally 4 °C).

4.3 Sample Material

Tap water from GAB Analytik, Pforzheim/Germany and river water from the "Nagold" at Werderbrücke, Pforzheim/Germany were used as matrix for method validation.
The samples were stored in a refrigerator at nominally 4 °C. Chemical and physical-chemical parameters were determined at CIP Pforzheim (non-GLP).

Parameter	Tap water	River water	Method of Determination
Appearence	colourless	brownish	sensory
pH (at 20°C)	7.75	8.22	DIN 38404-C 5
Specific electric conductivity (at 20°C, µS/cm)	442	708	DIN EN 27888 (C 8)
Total hardness (mmol/L)	13.4	15.6	complexometric titration
Spectral absorption coefficient (at 254 nm, m^{-1})	0.98	4.7	DIN 38404-C3
Dissolved organic carbon (mg C/L)	0.86	3.62	DIN EN 1484 (H 3)

5 Procedure for Captan Determination

5.1 Equipment

Glass bottles (500 mL or 1000 mL)
Volumetric flasks, 20 mL and 10mL
Apparatus for solid phase extraction including pressure gauge (SPE manifold)
PTFE connecting tubes
Magnetic stirrer and bars
Laboratory standard glassware and equipment
Gas chromatograph with mass selective detector

5.2 Reagents

Acetone, GC grade (Merck No. 1.00012)
n-Hexane, GC grade (Merck No. 1.04369)
Citral, p a (Merck No. 8.02489)
Acetic acid, conc., per analysis (Merck No. 1.00063)
Bond Elut PPL (200 mg, 3 mL, Varian No. 12105005)
LiChrolut (200 mg, 3 mL, Merck No. 1.19870.0001)

5.3 Extraction

Water samples were divided into aliquots of 500 mL and transferred into 500 mL glass bottles. In case of recovery experiments, fortification standard solution was added at this step (see caption 7.1). To the sample 0.2 mL acetic acid was added. The sample was homogenized for half a minute using a magnetic stirrer.

SPE cartridges were conditioned with each 3 mL acetone, followed by 2 x 3 mL demineralized water.

The water samples were attached to a vacuum manifold using PTFE connectors and passed through the SPE cartridges at about 1-2 drops per second.

After complete sampling, the cartridges were dried by sucking air through them for 5 minutes using the vacuum manifold.

Volumetric flasks (2 mL) were placed under the cartridges and captan was eluted with each 2.5 mL acetone. The solvent was evaporated to dryness using a gentle nitrogen stream and reconstituted in 1 mL of n-hexane. To the sample 10 µL citral was added. The sample was then determined by GC/MS.

5.4 Gas Chromatographic Analysis

Gas chromatograph:	Agilent HP 6840N
Column:	Varian CP-Sil 8 CB Low Bleed/MS (DB-5 equivalent), ≈15 m x 0.25 mm i.d., 0.25 µm film thickness
Carrier gas:	Helium, 1 mL/min
Injection:	2 µL pulsed splitless at 200 °C, 150 kPa for 1 min
Temperature program:	90 °C, 20 °C/min to 130°C, 10 °C/min to 195 °C, 5 °C/min to 205 °C, 30 °C/min to 310 °C, hold 5 min
Detector:	Agilent 5973 N mass selective detector in SIM mode
Temperatures:	Transfer line: 280 °C Ionizer: 230 °C (EI+, 70 eV) Quadrupole: 150 °C
SIM masses:	m/z = 264, 266, 114
Retention time:	≈9.6 min
Quantification:	Peak area, external standard calibration.

At the beginning of the sequence standard solutions in n-hexane were injected for constructing a calibration graph. Injections of samples were interspersed with injections of standard solutions to provide a continuous check on the response of the GC/MS system.

5.5 Calculation

Residues were calculated by the following equation:

$$R = \frac{c \cdot V_{End}}{V}$$

R Residue (µg/L)

c Captan concentration in final extract, as calculated from the peak areas of the sample vs. external standards calibration. Corrected by interspersed standard solutions and observed matrix effects (µg/mL).

V_{End} Final volume of extract (1 mL)

V Sample volume (0.5 L)

Captan recovery from surface water (0.1 µg/L, recovery sw 0.1-6):

$$R = \frac{0.047 \cdot 1}{0.5} = 0.094 \text{ µg/L} = 94 \text{ \%}$$

6 Deviations from the Study Plan

The study was performed according to the study plan dated 07 February 2005 amended on 01 March 2005 with the following deviation:

- For stabilization of captan a certain amount of acetic acid was added into the sample

The deviation has no negative impact on this study. This report reflects the conduct of this study.

7.3 Limit of Quantification

The limit of quantification was defined as the lowest fortification level with mean recoveries ranging between 70 % and 110 % at a relative standard deviation not exceeding 20 %, and residues in blanks not exceeding 30 %. These conditions are fulfilled at the 0.1 µg/L fortification level.

7.4 Repeatability

The relative standard deviations (RSD) per fortification level are within the guideline requirements (≤ 20 % RSD per fortification level) and indicate an good repeatability of the method for water.

7.5 Linearity

The calibration graph was linear and forced to origin within the range from 15 ng/mL to 750 ng/mL (corresponding 0.03 µg/L to 1.5 µg/L) captan ($y = 1048x$) and a correlation coefficient of $r^2 = 0.999$ (see appendix).

7.6 Specificity

A specific detection system was used (GC/MS). The retention time of the analyte in standard solutions matched the retention time in extracts from recovery samples. Three specific mass fragments above m/z = 100 were used for specification of captan. At the retention time of captan minor peak interferences were observed on quantifier mass fragment 114. The analytical method can be therefore regarded as specific for captan parent compound.

7.7 Storage Stability of Captan in Final Sample Extracts

Sample extracts were stored up to 5 days until final analysis by GC/MS. The storage stability was tested by comparison of one prepared matrix matched standard solution containing 50 µg/mL, prepared on 15 March 2005, with a matrix matched standard solution prepared on 11 March 2005.

Within the storage period no significant degradation (< 5%) of captan was found in samples from surface water. Therefore, captan can be regarded as stable in the final water sample extracts under the described conditions.

7.8 Matrix Effects

To check the matrix effect of sample extracts on GC/MS a matrix matched standard solution containing 50 ng/mL and 500 ng/mL were prepared from each matrix and measured versus standard solutions in n-hexane. Following matrix effects were observed:

Table 2: Matrix Effects

Matrix	Standard 0.05 µg/mL	Standard 0.5 µg/mL
Tap water	< 10 %	16 %
Surface water	< 10 %	20 %

Therefore the measured captan concentration from recovery samples at 10 x LOQ were corrected by observed matrix effects.

8 Discussion and Conclusions

An analytical method for determination of captan in surface water (river water) and drinking water (tap water) has been successfully validated.

For stabilization of captan in samples acetic acid was added. As analyte protectant for GC analysis a certain amount citral was added.

Validation of the method demonstrated that it was specific to captan with a quantification limit of 0.1 µg/L. The results of this study indicate that the recovery efficiency and repeatability were within the limits of 70 % - 110 % for mean recovery and < 20 % RSD at each fortification level. Interferences on quantification mass fragment from the control matrix used to validate the quantification limits were not observed. Due to GC/MS detection in SIM mode with three characteristic fragment ions with m/z > 100, analysis is considered to be specific for captan parent compound. Only minor interferences on qualification mass 114 were observed.

In consequence, the analytical method fulfills the requirements of guideline SANCO/825/00 rev. 7.

9 References

EUROPEAN COMMISSION, DIRECTORATE GENERAL HEALTH AND CONSUMER PROTECTION (2004): Guidance document on residue analytical methods. SANCO/825/00, rev. 7, 17/03/2004

10 Archiving

For the periods demanded by the principles of GLP the following documents and materials will be archived:

- Study plan, raw data, comments of the sponsor on the draft report and the final report.
- All documentation generated by the Quality Assurance Unit.
- A sample of the test substance.

All documents and materials will be stored in the archives of GAB Biotechnologie GmbH & GAB Analytik GmbH. The premises for storing the documents and materials are settled according to the principles of Good Laboratory Practice in the organization of the testing facility.