1. INTRODUCTION

Background and Objective:

The objective of this study is to develop and to validate an analytical method for the determination of thiophanate–methyl and carbendazim in drinking (tap), ground (well) and surface (river or pond) water to achieve a limit of quantitation (LOQ) of 0.05 µg/L.

Data Requirements and Guidelines:

EC Guidance document on residue analytical methods (SANCO/825/00 rev. 8.1, 16-Nov-10) and OECD Guidance Document on Pesticide Residue Analytical Methods (ENV/JM/MONO(2007)17, 13-Aug-07.

Method Principles and Method Validation:

Drinking, ground and surface water were acidified before analysis. Determination of final sample volumes was conducted with LC-MS/MS with direct injection.

For method validation the drinking, ground and surface water specimens were fortified with separate spike solutions (5 replicates per fortification level) at 0.05 µg/L (LOQ) and at 0.50 µg/L (10xLOQ) with thiophanate-methyl and carbendazim. Additionally two untreated blank control specimens per water type were analysed.

2. EXPERIMENTAL

2.1 Test System

Drinking (Tap) Water

Water was collected from a PTRL Europe laboratory tap located in Ulm, in Southern Germany. The appearance of the water was clear and without any odor. The water was characterized for physical and chemical properties as follows: pH 7.72, total water hardness: 2.43 mmol/L (Deutsche Härtegrade, 13.6°d) by accredited Institute Alpha (Ulm, Germany following common DIN or EN guidelines and methods, non-GLP).

Ground (Well) Water

Water was collected on 12-Jul-12 from Herbrechtingen, in Southern Germany. The water was characterized for physical and chemical properties by accredited Institute Alpha (Ulm, Germany following common DIN or EN guidelines and methods), resulting in the following (non-GLP):

Total water hardness:	3.04 mmol/L (Deutsche Härtegrade, 17.1°d)
TOC (total organic carbon, EN 1484:1997):	2.6 mg/L
DOC (diluted organic carbon, EN 1484: 1997):	1.3 mg/L
pH (DIN 38 404-C5):	7.91

Surface (River) Water

Water was collected on 12-Jul-12 from the Brenz River in Herbrechtingen, located in Southern Germany. The water was characterized for physical and chemical properties by accredited Institute Alpha (Ulm, Germany following common DIN or EN guidelines and methods), resulting in the following (non-GLP):

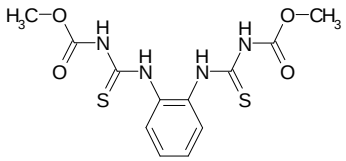
Total water hardness:	1.27 mmol/L (Deutsche Härtegrade, 7.2°d)
TOC (total organic carbon, EN 1484:1997):	0.82 mg/L
DOC (diluted organic carbon, EN 1484: 1997):	0.70 mg/L
pH (DIN 38 404-C5):	7.96

Water was stored at room temperature in the dark when not used.

2.2 Analytical Test and Reference Item

The following standards provided by the laboratory Sigma Aldrich (thiophanate-methyl) and laboratory Dr. Ehrenstorfer (carbendazim, see Appendix 1) were used as test / reference items:

Thiophanate-methyl

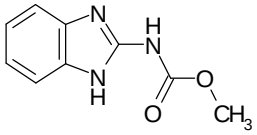


Empirical Formula: $C_{12}H_{14}N_4O_4S_2$ Molar Mass: 342.4 g/mol

IUPAC name: Dimethyl 4,4'-(o-phenylene)bis(3-thioallophanate)

CAS number: [23564-05-8] Purity: 99.3 %

Carbendazim



Empirical Formula: $C_9H_9N_3O_2$ Molar Mass: 191.2 g/mol

IUPAC name: Methyl benzimidazol-2-ylcarbamate

CAS number: [10605-21-7] Purity: 98.5 %

2.3 Analytical Method

2.3.1 Apparatus

2.3.1.1 Laboratory Equipment

XP205DR balance, Mettler Toledo.
Transsonic 700 bath, Elma Hans Schmidbauer.
Vortex mixer REAX top, Heidolph.
Typical glassware and laboratory equipment.
All glassware was cleaned in a laboratory dishwasher and air-dried before use.

2.3.1.2 LC-MS System

Agilent 1200 SL Series HPLC system (vacuum solvent degasser, binary HPLC pump, column oven), and CTC Analytics HTC-Pal Autosampler.
Column: Agilent Zorbax, 3.5 µm particle size, 75 mm length, 4,6 mm i.d. Pre-column: Phenomenex C_{18}, 4 mm length, 3.0 mm i.d.

Applied Biosystems MDS Sciex API 5500 triple quadrupole LC-MS/MS system with Turbo IonSpray ESI source. Analyst 1.5.2 Instrument control and data acquisition software.

2.3.2 Solvents and Chemicals

Millipore Water (PTRL Europe)
Acetonitrile and Methanol, HPLC Grade (≥ 99.9 %), Promochem.
Formic acid (98-100%), Sigma Aldrich.
Glacial acetic acid (100%), Merck.

2.3.3 Preparation of Standard Solutions

Stock solutions of thiophanate-methyl and carbendazim were prepared in acetonitrile/methanol (7/3, v/v) containing 0.1% acetic acid, such as described in the following table:

Substance name	Weight [mg]	Dissolve in [mL]	Obtain [mg/mL] (*)
Thiophanate-methyl (purity 99.3 %)	20.15, 20.16	20	1.0
Carbendazim (purity 98.5 %)	10.14, 10.15	50	0.20

(*): Purity taken into account.

For each analyte, fortification solutions with concentrations of 5.0 µg/mL, 0.05 µg/mL and 0.005 µg/mL were prepared in acetonitrile containing 0.1 % formic acid, by accurate dilution of the stock solutions. Calibration solutions for both analytes were prepared by volumetric dilution in water containing 0.1% formic acid to obtain concentrations ranging from 0.010 ng/mL to 10 µg/mL.
Calibration solutions in extracts from blank control samples used to show matrix effect were prepared by accurate dilution of a selected calibration solution in solvent to obtain the concentration of 0.10 ng/mL.

All standard solutions were stored frozen (at or below -18°C) in amber glass bottles when not in use.

2.3.4 Stability of Standard Solutions and Extracts

Stability of solutions is proven by comparing two calibration solutions diluted from an old and a freshly prepared stock solution (refrigerated storage for seven days; see Table 4).

Deviation was < 15 %.

2.3.5 Sample Analysis

1. Place 1.0 mL of drinking, ground or surface water into an autosampler vial.
2. Fortify the water sample, with 0.010 mL of 5.0 ng/mL or with 0.010 mL of 50 ng/mL fortification solution for 0.05 µg/L and 0.50 µg/L, respectively.
3. Acidify the sample with 10 µL of water containing 10% of formic acid (final concentration formic acid = 0.1%).
4. Vortex the sample.
5. Analyse the sample by LC-MS/MS.

2.4 LC-MS/MS Analysis

Specimens and calibration solutions in solvent were analyzed by liquid chromatography with tandem mass spectrometric detection (LC-MS/MS):

LC System	Agilent 1200 SL HPLC system (vacuum solvent degasser, binary HPLC pump, column oven), and CTC Analytics HTC-Pal Autosampler
LC Column	Agilent Zorbax column: Length: 75 mm, i.d.: 4.6 mm, particle size: 3.5 µm, oven temp.: 35°C.
LC Injection Volume	100 µL.
LC Method	Solvent A: Water containing 0.1 % formic acid Solvent B: Acetonitrile containing 0.1 % formic acid Program: Time (min) / Flow rate (mL/min) / % A / % B 0.00 / 0.600 / 80 / 20 3.00 / 0.600 / 0 / 100 6.00 / 0.600 / 0 / 100 6.10 / 0.600 / 80 / 20 10.00 / 0.600 / 80 / 20
Retention time	≈ 2.3 min for carbendazim and ≈ 4.6 min for Thiophanate-methyl
MS/MS System	Applied Biosystems MDS Sciex API 5500 triple quadrupole LC-MS/MS system with TurboIonspray (ESI) source.
Ion Source Conditions ESI Positive Polarity	Source temperature: 600°C Gas supply (GS 1): 90 (arbitrary units) Gas supply (GS 2) 0 (arbitrary units) Curtain gas: 12 (arbitrary units) CAD gas: Medium Entrance potential: 10 V IonSpray voltage: 5500 V Resolution: Q1: Unit, Q3: Unit

MS/MS Conditions	Thiophanate-methyl	
	MS/MS transition for quantification:	343 *m/z* > 151 *m/z*
	Collision energy (CE):	30 V
	Cell exit potential (CXP):	13 V
	Dwell time:	300 ms
	Declustering potential (DP):	107 V
	MS/MS transition for confirmation:	343 *m/z* > 192 *m/z*
	Collision energy (CE):	30 V
	Cell exit potential (CXP):	13 V
	Dwell time:	500 ms
	Declustering potential (DP):	107 V
	Carbendazim	
	MS/MS transition for quantification:	192 *m/z* > 160 *m/z*
	Collision energy (CE):	25 V
	Cell exit potential (CXP):	9 V
	Dwell time:	100 ms
	Declustering potential (DP):	88 V
	MS/MS transition for confirmation:	192 *m/z* > 132 *m/z*
	Collision energy (CE):	43 V
	Cell exit potential (CXP):	7 V
	Dwell time:	250 ms
	Declustering potential (DP):	88 V

See Figure 27 and Figure 28 for the product ion spectra of thiophanate-methyl and carbendazim.

The quantitative determination was carried out by external standardization using calibration standards in solvent. Calibration functions ranging from 0.01 to 1.0 ng/mL were used to evaluate the final sample volumes (exemplified in Figure 1 to Figure 4).

Representative LC-MS/MS ion chromatograms of calibration solutions in solvent and of final sample volumes of fortified and control specimens are presented in Figure 5 to Figure 26.

2.5 Calculations

Recovery results derived from LC-MS/MS analysis and calculations are shown in details in Table 1 to Table 3.

The following equation was used to calculate the individual residues R in µg/L:

$R = c_{End} \times (V_{End} / V_{Sample})$

$= c_{End} \times$ Multiplier M

R: Analyte residue in µg/L.

c_{End}: Concentration of analyte in final sample volume, in ng/mL.
(where multiple injections were evaluated: mean).

V_{Sample}: Water volume: 1.0 mL.

V_{End}: Final sample volume: 1.0 mL.

Recoveries (Rec.) were calculated for the fortified specimens as follows:

$Rec. = (R / R_{fortified}) \times 100\ \%$

The calculation is exemplified with the drinking water specimen P2681-84 fortified at 0.05 µg/L (LOQ). The final sample volume was examined by LC-MS/MS in run file

P2681#035 to give a final concentration C_{End} of 0.05 ng/mL for 343 *m/z* -> 151 *m/z* and 0.0546 ng/mL for 343 *m/z* -> 192 *m/z*, respectively. The following calculation is demonstrated for the fragment ion 151 m/z:

Thus:

R = c_{End} x (V_{End} / V_1)
= c_{End} x Multiplier M
= 0.05 ng/mL x (1.0 mL / 1.0 mL)
= 0.05 ng/mL x 1.0
= 0.05 ng/mL or µg/L

Rec. = (R / $R_{fortified}$) x 100 %
= (0.05 µg/L / 0.05 µg/L) x 100 % = 100 %

Calculations were performed with full precision by computer software (Excel). Thus slight discrepancies may arise when recalculated using a pocket calculator.