

## 2. EXPERIMENTAL

### 2.1 Test System

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):

#### 2.1.1 Drinking (Tap) Water

Drinking (tap) water was collected on 13-Jan-14 from Ulm, Southern Germany.

Total water hardness (EN 11885:2009):	3.10 mmol/L (calculated from Mg and Ca) (Deutsche Härtegrade:17.4°d)
TOC (total organic carbon, EN 1484:1997):	0.75 mg/L
DOC (dissolved organic carbon, EN 1484: 1997):	0.75 mg/L
pH (DIN 38 404-C5):	7.23
Silt content (EN 872 Whatman GF 6):	< 0.1 mg/L
Electric conductivity (at 25°C, EN 27888:1993)	766 µS/cm

#### 2.1.2 Surface (River) Water

Surface water was collected on 09-Sep-13 from the River Danube in Ulm, Southern Germany.

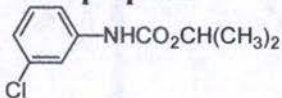
Total water hardness (EN 11885:2009):	3.20 mmol/L (calculated from Mg and Ca) (Deutsche Härtegrade:17.9°d)
TOC (total organic carbon, EN 1484:1997):	1.9 mg/L
DOC (dissolved organic carbon, EN 1484: 1997):	1.7 mg/L
pH (DIN 38 404-C5):	8.16
Silt content (EN 872 Whatman GF 6):	3.1 mg/L
Electric conductivity (at 25°C, EN 27888:1993)	584 µS/cm

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

### 2.2 Analytical Test and Reference Item

An analytical standard of chlorpropham purchased commercially from Sigma Aldrich (see APPENDIX 1 for Certificate of Analysis) was used as test / reference item:

#### Chlorpropham



IUPAC name: Isopropyl 3-chlorocarbanilate

Molecular formula: C<sub>10</sub>H<sub>12</sub>ClNO<sub>2</sub>

Molecular mass: 213.7 g/mol

The analytical standard was stored at room temperature when not use.



## 2.3 Analytical Method

### 2.3.1 Apparatus

#### 2.3.1.1 Laboratory Equipment

Mettler-Toledo XP205DR analytical balance for analytical standard.

Vortex mixer REAX top, Heidolph.

Ultrasonic bath USC 600T, VWR international bvba.

Typical glassware and laboratory equipment.

All the glassware was cleaned in a laboratory dishwasher and air-dried before use.

#### 2.3.1.2 LC-MS/MS System

AB Sciex API 5500 triple quadrupole LC-MS/MS system, equipped with TurboIonSpray ESI source, Agilent 1290 Infinity Series HPLC system (vacuum solvent degasser, binary HPLC pump, column oven), and CTC Analytics HTC-Pal Autosampler, Analyst 1.6.2 Instrument control and data acquisition software.

Column:

Supelco Ascentis Express C<sub>18</sub>, 2.7 µm particle size, 50 mm length, 2.1 mm i.d.

Pre-column: Phenomenex C<sub>18</sub>, 4 mm length, 3.0 mm i.d.

### 2.3.2 Solvents and Chemicals

Methanol, Promochem, HPLC grade.

Acetonitrile, Promochem, HPLC grade.

Millipore water, supply at PTRL Europe.

Formic acid, Sigma Aldrich, 98-100%.

### 2.3.3 Preparation of Standard Solutions

A stock solution of chlorpropham was prepared in acetonitrile as follows e.g.:

Substance name	Weight [mg]	Dissolve in [mL]	Obtain [mg/mL]
Chlorpropham (purity 99.7 %)	10.06	10	1.0

Fortification solutions of chlorpropham with concentrations of 10, 0.10 and 0.010 µg/mL were prepared in acetonitrile by accurate dilution of the stock solution.

Calibration solutions for chlorpropham were prepared by volumetric dilution in acetonitrile to obtain a concentration of 10 µg/mL and in water containing 0.1% formic acid to obtain a concentration of 0.10 µg/mL (intermediate solutions).



The intermediate solutions were diluted in water containing 0.1% formic acid to obtain concentrations from 0.030 to 10 ng/mL of chlorpropham.

For preparation of matrix-matched standards calibration solutions in solvent were diluted in drinking and surface water (both acidified at 0.1% formic acid) resulting in concentrations of 0.10 and 1.0 ng/mL.

All standard solutions were stored refrigerated when not in use.

#### 2.3.4 Stability of Standard Solutions and Extracts

Stability of stock solutions is proven for at least 11 days under refrigerated conditions by comparing two solutions diluted from an old and a freshly prepared stock solution. Stability of calibration solutions is proven for at least 6 days under refrigerated conditions (see Table 4).

Selected fortified water samples were re-injected after at least 5 days of refrigerated storage (as shown in Table 5). The results obtained were lower by more than 20 %, thus indicating that stability of the analyte in water samples is limited.

#### 2.3.5 Effects of Matrix on Analyte Response

No significant effects of matrix ( $\leq 20\%$  enhancement or suppression) on LC-MS/MS response were observed (see Table 3).

#### 2.3.6 Residue Analysis

1. 1.0 mL of drinking or surface water was dosed into an autosampler vial.
2. 10  $\mu$ L of the corresponding fortification solution was added, if necessary.
3. 10  $\mu$ L of 10% formic acid in water was added to each sample.
4. The sample was mixed using a vortexer.
5. LC-MS/MS analysis.

### 2.4 LC-MS/MS Analysis

The final extracts were analyzed by liquid chromatography with tandem mass spectrometric detection (LC-MS/MS).

#### LC Conditions

LC System	Agilent 1290 Infinity HPLC system (vacuum solvent degasser, binary HPLC pump, column oven), and CTC Analytics HTC-Pal Autosampler.
LC Column	Supelco Ascentis Express C <sub>18</sub> column: Length: 50 mm, i.d.: 2.1 mm, particle size: 2.7 $\mu$ m.
LC Injection Volume	40 $\mu$ L.



LC Method	Solvent A:	Water containing 0.1% of formic acid		
	Solvent B:	Methanol containing 0.1% of formic acid		
	Mobile Phase Composition:			
	Time (min)	Flow rate (mL/min)	% A	% B
	0.00	0.400	80	20
	2.00	0.400	80	20
	2.10	0.400	0	100
	5.00	0.400	0	100
5.10	0.400	80	20	
8.00	0.400	80	20	
Retention Time	Chlorpropham: approx. 3.3 min.			
Column oven temperature	35 °C			

### MS Conditions

The  $[M+H]^+$  ion of chlorpropham at 214 m/z was used as parent ion for MS/MS detection. The MS/MS transition to the daughter ion at 172 m/z was used for quantification of the analyte. A 2<sup>nd</sup> MS/MS transition (214 m/z → 126 m/z) was used for quantitative confirmation.

MS System	Applied Biosystems MDS Sciex API 5500 triple quadrupole LC/MS/MS system with Turbolonspray (ESI) source.	
Ion Source Conditions ESI Positive Polarity	Source temperature:	400°C
	Gas supply (GS 1):	40 (arbitrary units)
	Gas supply (GS 2):	60 (arbitrary units)
	Curtain gas:	12 (arbitrary units)
	CAD gas:	Medium
	Entrance potential:	10 V
	IonSpray voltage:	4500 V
	Resolution Q1:	unit
	Resolution Q3:	unit
MS/MS Conditions	MS/MS transition for quantification:	214 m/z > 172 m/z
	Collision energy (CE):	13 V
	Cell exit potential (CXP):	16 V
	Dwell time:	350 ms
	Declustering potential (DP):	66 V
	MS/MS transition for confirmation:	214 m/z > 126 m/z
	Collision energy (CE):	33 V
	Cell exit potential (CXP):	12 V
	Dwell time:	350 ms
	Declustering potential (DP):	66 V

Figure 1 shows the product ion spectrum for chlorpropham. The quantitative determination was carried out by external standardization using calibration solutions in solvent. Calibration functions ranging from 0.030 to 10 ng/mL were used to evaluate the extracts.

## 2.5 Calculations

When water samples are analysed by direct injection (DI-)LC/MS/MS no recovery calculations are required (according to SANCO/825/00 rev. 8.1). Concentration C used for storage stability calculations are calculated by the LC/MS software based on external calibration.