5 EXPERIMENTAL DETAILS

5.1 Reference item(s)

Name	Chlorpropham
CAS Number	101-21-3
ANADIAG Ref.	3638
Supplier	Sigma Aldrich
Batch No.	SZE8315X
Purity (%) *	99.7
Expiry date	10/11/2015
Storage conditions	≈ -18°C

* See certificate of analysis in Appendix I.

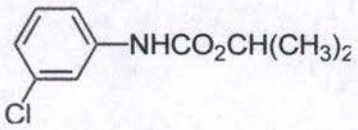
Chlorpropham

Chemical Abstracts Name: 1-methylethyl (3-chlorophenyl)carbamate

Molecular formula: $C_{10}H_{12}ClNO_2$

Molecular weight: 213.7 g/mol

Structural formula:



5.2 Origin of the specimens

The test system consisted of tap water sample at ANADIAG laboratory.

5.3 Specimen preparation

The specimens were prepared according to ANADIAG SOP PG 0115.

About 1 L of tap water was sampled and hand homogenised. The amount required by the analytical method was measured from this homogeneous matrix.

5.4 Materials

5.4.1 Apparatus

- Usual laboratory glassware
- Vortex

5.4.2 Reagents

- HPLC Acetonitrile, milli-Q H_2O, HPLC MeOH
- formic acid

5.4.3 Reagents and mixture preparation

Formic acid 10%

Approximately 8 mL milli-Q H_2O was transferred into a 10 mL volumetric flask. 1 mL formic acid was added then made up to the mark with milli-Q H_2O.

Milli-Q H_2O + 0.1% formic acid

Approximately 80 mL milli-Q H_2O was transferred into a 100 mL volumetric flask. 0.1 mL formic acid was added then made up to the mark with milli-Q H_2O.

Mobile phase A: milli-Q H_2O + 0.1% formic acid
1L milli-Q H_2O was transferred into a 1 L bottle. 1 mL formic acid was added before mixing.

Mobile phase B: HPLC methanol + 0.1% formic acid
1L HPLC methanol was transferred into a 1 L bottle. 1 mL formic acid was added before mixing.

5.5 Extraction and Clean-up

1 mL of drinking water was dosed into an autosampler vial and spiked if necessary (10 µL of spiking solution).

10 µL of formic acid at 10% were added and the autosampler was mixed using a vortexer before analysis by LC-MS/MS.

5.6 Standard solutions

5.6.1 Stock solution

Chlorpropham

A standard stock solution at approximately 1 mg/mL was prepared by accurately weighing 10 mg of chlorpropham analytical standard into a 10 mL volumetric flask and bringing to volume with acetonitrile.

5.6.2 Spiking solutions

A secondary solution at 10 µg/mL was prepared by dilution of the stock solution with acetonitrile.

Spiking solutions at 10 and 100 ng/mL were prepared by dilution of the secondary solution with acetonitrile.

Fortifications were performed by adding known amounts of chlorpropham to control specimens just prior to the extraction step according to SOP PG 0119. These fortified specimens were analysed along with control specimens.

5.6.3 Calibration solutions

An intermediate solution at 100 ng/mL was prepared by dilution of the secondary solution at 10 µg/mL with milli-Q H_2O + 0.1 % formic acid.

Calibration solutions ranging from 0.03 to 10 ng/mL were prepared by dilution of this intermediate solution with milli-Q H_2O + 0.1% formic acid.

5.6.4 Matrix-matched calibration solutions for matrix-effect testing

Several control specimens of each matrix were extracted to prepare "control extracts".

Matrix-matched calibration solution at 1 ng/mL was prepared by dilution of the intermediate solution at 100 ng/mL with the control extract.

5.6.5 Calibration curves

Aliquots of the calibration solutions were injected into the analytical system using the same conditions as the specimens.

Peak areas of chlorpropham obtained from chromatograms were plotted versus concentration and the calibration functions were determined by least square fit.

Number and concentrations of standards used, as well as acceptability criteria are described in SOP No. PG 0118. According to this SOP, the correlation coefficient for a curve (r) should be ≥ 0.990 for the calibration to be acceptable.

5.7 Analytical conditions

Analytical Conditions
Xevo-TQMS : LC-MS/MS

No. MA_1035-01

Apparatus	XEVO LC /MS /MS

Column

Description	Ascentis Express C18	Supplier	Supelco	Particles	1.7 µm
Internal diam. x length	50 X 2,1 mm	Supplier reference	53822-U	Temperature	35 °C
Development Column ANADIAG Number	196	Stationary Phase	C18	Comment	-

Mobile phase

A =	H_2O + 0.1% formic acid	C =	-
B =	MeOH + 0.1% formic acid	D =	-

Sample temperature	15 ° C

Elution

Elution	Time	Flow	Composition (%)				Curve	Elution	Time	Flow	Composition (%)				Curve
	min	mL/min	A	B	C	D	(type)		min	mL/min	A	B	C	D	(type)
Pg1	0.00	0.4	80	20	-	-		Pg5	5.10	0.4	80	20	-	-	6
Pg2	2.00	0.4	80	20	-	-	6	Pg6	8.00	0.4	80	20	-	-	-
Pg3	2.10	0.4	0	100	-	-	6	Pg7	-	-	-	-	-	-	-
Pg4	5.00	0.4	0	100	-	-	6	Pg8	-	-	-	-	-	-	-

Detector

IONISATION mode*	ES x	APCI
Polarity*	Pos x	Neg

*make a cross in the right choice

Active ingredient(s)	Cone voltage	Collision Energy	Dwell time (ms)	TRANSITION 1	TRANSITION 2	RT (min.)
Chlorpropham	10	10	350	214.1 > 172.1	-	≈ 3.2
	10	25	350	-	214.1 > 126.0	

Date of application of analytical conditions: 14/11/2014

Study	B4032	Column ANADIAG number	196
Matrix	Drinking water	Retention time	Chlorpropham: ≈ 3.2 min.
Sample temperature	+15°C	Injected volume	90 µL

5.8 Calculations

Extracts were injected into the analytical system. The peak area was measured for chlorpropham and residue concentrations (ng/mL) were calculated from the appropriate standard curve, using the average response factor of standard solutions bracketing sample extracts (as the factor was not stable enough according to SOP PG0118).

Chlorpropham concentration (ng/mL) in extract was determined as follows:

Conc. (ng/mL) = Peak area / FRe
(FR_e= response factor in extract calculated with the mean of the response factor of two standards bracketing samples.)

The amount of chlorpropham in the specimens was determined using the following equation:

$$\textbf{Amount found (µg/L)} = \frac{\textbf{Conc. x V x d}}{\textbf{M x \% aliquot purified}}$$

Calculations were based on real values. Intermediate values were not rounded.

Where: Conc. Concentration in extract (ng/mL)
V Final volume of the extract (mL)
d Dilution factor
M Sample volume (mL)

5.8.1 Calculation of recoveries

$$\textbf{\% recovery} = \frac{\textbf{µg/L found in fortified sample - µg/L found in control sample*}}{\textbf{µg/L added to fortified sample}} \textbf{ x 100}$$

* Amount in control is deducted if above the LOD level.

5.8.2 Example of calculation

Example of calculation with response factor

Specimen No. B4032 01 01 CA (file name B4032-024, see detailed data in Appendix II).

Conc. (ng/mL) = 272 / 3.0950E+02 = 0.088 ng/mL

$$\textbf{Residue (µg/L)} = \frac{\textbf{0.088 ng/mL x 1.0 mL x 1}}{\textbf{1.0 mL x 100 \%}} \textbf{ = 0.088 µg/L}$$

$$\textbf{\% recovery} = \frac{\textbf{0.088 µg/L – 0.00 µg/L}}{\textbf{0.101 µg/L}} \textbf{ x 100 = 87.1\%}$$

5.9 Deviation(s)

No deviation was recorded during this study.