FINAL REPORT

Independent Laboratory Validation of an Analytical Method for the Determination of Residues of Tri-allate and its Metabolite TCPSA in Drinking Water

GUIDELINES

Regulation (EC) No 1107/2009
Guidance document SANCO/825/00 rev.8.1 of November 16, 2010, European Commission

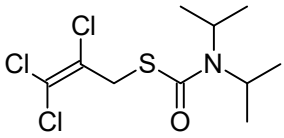
2 Study Objective

The study objective was to perform an independent laboratory validation (ILV) of analytical methods developed at Laboratorio de Análisis de Residuos de Plaguicidas, Universitat Jaume I, Castellón / Spain in 2016 (López Benet, F., study code 268-16) for the determination of residues of Tri-allate and TCPSA in drinking water according to guideline SANCO/825/00 rev.8.1 using HPLC-MS/MS.

3 Material and Methods

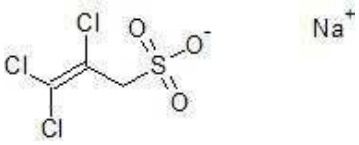
3.1 Test and Reference Items

3.1.1 Tri-allate

Name:	Tri-allate
Chemical name (IUPAC):	S-2,3,3-trichloroallyl di-isopropylthiocarbamate
CAS Registry No.:	2303-17-5
Structural formula:	
Molecular formula:	$C_{10}H_{16}Cl_3NOS$
Molecular Mass:	304.66 g/mol
CIP code:	10105
Supplier:	Sigma-Aldrich
Batch.:	SZBD301XV
Purity:	98.8 %
Date Certificate of Analysis:	12 November 2013
Recommended Storage Conditions:	Refrigerated
Storage Conditions at CIP:	Refrigerated
Date of Expiry:	28 October 2018

All specifications of purity and composition were provided by the supplier.

3.1.2 TCPSA

Name:	TCPSA
Chemical name (IUPAC):	2,3,3-trichloro-prop-2-en-sulfonic acid, sodium salt
CAS Registry No.:	65600-61-5
Structural formula:	
Molecular formula:	$C_3H_2Cl_3O_3S$ Na
Molecular Mass:	247.46 g/mol (free acid: 225.48 g/mol)
CIP code:	10106
Supplier:	Synpura
Batch.:	SP17-100-1-1 & SP17-100-1-2
Purity:	99.4%
Date Certificate of Analysis	09 February 2017
Recommended Storage Conditions:	Ambient
Storage Conditions at CIP:	Refrigerated
Date of Expiry:	09 February 2019

All specifications of purity and composition were provided by the supplier.

3.2 Analytical Procedure

3.2.1 Specimen Origin

Drinking water was taken from a local water supplier in Pforzheim, Germany. The parameters of the used water sample are given in Table 1:

Table 1: Typical parameters of the used drinking water

	drinking water
Origin	Drinking Water Pforzheim / Germany
pH	7.66
Conductivity (25°C) [µS/cm]	423
TOC [mg/L]	0.7
Total hardness [mmol/L]	2.42

All parameters were analysed according to DIN EN ISO/IEC 17025:2005 (non-GLP).

3.2.2 Specimen Preparation and Storage

The drinking water sample was stored refrigerated (≤8°C) until start of analysis and was homogenised by shaking before use.

3.3 Outline of the Method

Residues of Tri-allate were analysed after dilution with HPLC water. Residues of TCPSA were analysed after filtration. Analysis was performed by HPLC coupled with tandem mass spectrometry (HPLC-MS/MS) for both analytes.

A detailed description of the method is given in chapter 3.6.

Representative chromatograms are presented in Appendix 3.

3.3.1 Hazards or Precautions

For conduct of this method the German guidelines for laboratories „Working Safely in Laboratories, Basic Principles and Guidelines“ or comparable guidelines in other countries are to be observed. The following chemicals are used, which are classified by the hazardous material regulations according to Regulation (EC) No 1272/2008 [EU-GHS/CLP].

The pertinent safety instructions must be observed when working with all compound mentioned in this method (e.g. hazard (H) and precautionary (P) statements).

3.4 Equipment and Apparatus

- Adjustable pipettes (Eppendorf/Germany or Thermo Labsystems/Finnland)
- Microliter syringes
- HPLC Autosampler vials, 1.8 mL
- HPLC with MS/MS detection (Dionex Ultimate 3000 with AB Sciex API 5500 QTRAP)
- Laboratory balance (Sartorius A 200 S)
- Standard laboratory glassware

3.5 Reagents and Materials

- Acetonitrile, HPLC gradient grade (LGC No.SO-9128-B025)
- Acetone, for residue analysis (LGC No. SO-1142-B025
- Water, HPLC grade (LGC No. SO-6795-B025)
- Methanol, Chromasol (Honeywell No. 34966-2.5L)
- Formic acid (Merck No.1.00264.1000)
- Acetonitrile, LCMS grade (LGC No.SO-9340-B010)
- Water, LCMS grade (LGC No.SO-9368-B010)
- PTFE syringe filters, 0.2 µm, 25 mm (Wicom No. WIC80120)

3.6 Sample preparation

3.6.1 Method

Tri-allate: 0.5 mL of water sample and 0.5 mL of HPLC water were transferred into an autosampler vial. Analysis was performed by HPLC-MS/MS monitoring two mass transitions.

TCPSA: An aliquot of the water sample was filtered through a 0.2 µm PTFE filter and transferred into an autosampler vial. Analysis was performed by HPLC-MS/MS using two different chromatographic columns (quantification: phenyl hexyl, confirmation: C18).

3.7 HPLC-MS/MS Conditions for Analysis of Tri-allate

HPLC-MS/MS:	Dionex Ultimate 3000 with AB Sciex API 5500 QTRAP
Column:	Phenomenex Luna C18(2), 150 mm x 2.0 mm, 5.0 µm (Part No. 00F-4252-B0)

Mobile phase:	A: Water +0.01% formic acid B: Methanol +0.01% formic acid			
	Time [min]	A [%]	B [%]	Flow [µL/min]
	0.00	60	40	350
	1.00	60	40	350
	10.0	5	95	350
	15.0	5	95	350
	15.1	60	40	350
	19.0	60	40	350

Gradient:	Linear
Column temp.:	30 °C
Interface:	ESI
Source polarity:	Positive
Ion Source:	Turbo Spray
Curtain gas	20 units
Collision gas:	Medium
Ion spray voltage:	5.5 kV
Interface temperature:	550 °C
GS1:	40 units
GS2:	70 units
Injection volume:	30 µL*
Retention time:	~ 14.4 min
Split:	-
Valve:	0.1-8 min waste; 8-16 min MS/MS; 16--19 min waste
Quantification:	Matrix-matched external standards

*In the primary method validation an injection volume of 15 µL has been used. In the current ILV the injection volume has been set to 30 µL due to the needs of the analytical instrumentation used within this study for determination of Tri-allate.

Mass spectrometer parameters:

Analyte Monitored	Ions Monitored m/z	DP [V]	EP [V]	CE [V]	CXP [V]	Dwell Time [ms]
Tri-allate	304 → 86	66	10	21	14	150
	304 → 128	66	10	19	14	150

The linearity was proven by injecting matrix-matched standard solutions in the range of 0.0125 µg/L to 2.5 µg/L for Tri-allate.

3.8 HPLC-MS/MS Conditions for Analysis of TCPSA using Phenyl-Hexyl Column (quantification)

HPLC-MS/MS:	Dionex Ultimate 3000 with AB Sciex API 5500 QTRAP
Column:	Thermo Betasil Phenyl-Hexyl, 150 mm x 2.1 mm, 3.0 µm (Part No. 73003-152130)

Mobile phase:	A: Water +0.01% formic acid B: Acetonitrile

Time [min]	A [%]	B [%]	Flow [µL/min]
0.00	90	10	300
8.00	10	90	300
11.0	10	90	300
11.1	90	10	300
16.0	90	10	300

Gradient:	Linear
Column temp.:	30 °C
Interface:	ESI
Source polarity:	Negative
Ion Source:	Turbo Spray
Curtain gas	20 units
Collision gas:	Medium
Ion spray voltage:	-4.5 kV
Interface temperature:	650 °C
GS1:	40 units
GS2:	80 units
Injection volume:	25 µL
Retention time:	~ 6.6 min
Split:	-
Valve:	0-1 min waste; 1-16 min MS/MS
Quantification:	Matrix-matched external standards

Mass spectrometer parameters:

Analyte Monitored	Ions Monitored m/z	DP [V]	EP [V]	CE [V]	CXP [V]	Dwell Time [ms]
TCPSA	223 → 80	-74	-10	-35	-7	150

The linearity was proven by injecting matrix-matched standard solutions in the range of 0.025 µg/L to 2.5 µg/L for TCPSA.

3.9 HPLC-MS/MS Conditions for Analysis of TCPSA using C18 Column (confirmation)

HPLC-MS/MS:	Dionex Ultimate 3000 with AB Sciex API 5500 QTRAP
Column:	Phenomenex Luna C18(2), 150 mm x 2.0 mm, 5.0 µm (Part No. 00F-4252-B0)

Mobile phase:	A: Water +0.01% formic acid B: Acetonitrile

Time [min]	A [%]	B [%]	Flow [µL/min]
0.00	90	10	500
6.00	10	90	500
11.0	10	90	500
11.1	90	10	500
16.0	90	10	500

Gradient:	Linear
Column temp.:	30 °C
Interface:	ESI
Source polarity:	Negative
Ion Source:	Turbo Spray
Curtain gas	20 units
Collision gas:	Medium
Ion spray voltage:	-4.5 kV
Interface temperature:	650 °C
GS1:	40 units
GS2:	80 units
Injection volume:	15 µL*
Retention time:	~ 6.6 min
Split:	-
Valve:	0-0.1 min waste; 0.1-16 min MS/MS
Quantification:	Matrix-matched external standards

*In the primary method validation an injection volume of 25 µL has been used. In the current ILV the injection volume has been set to 15 µL due to the needs of the analytical instrumentation used within this study for determination of TCPSA using a C18 chromatographic column.

Mass spectrometer parameters:

Analyte Monitored	Ions Monitored m/z	DP [V]	EP [V]	CE [V]	CXP [V]	Dwell Time [ms]
TCPSA	223 → 80	-74	-10	-35	-7	150

The linearity was proven by injecting matrix-matched standard solutions in the range of 0.025 µg/L to 2.5 µg/L for TCPSA.

3.10 Calculation of the Residues

Matrix-matched external standard solutions comparable to the concentration expected in specimens were injected before and after every 2-4 samples in each analytical sequence.

The residues (R) in [µg/L] of Tri-allate and TCPSA were calculated using external matrix-matched bracketing standards according to the following equations:

$$R = \frac{A_x \cdot C_{St,nominal}}{A_{St}} \cdot df$$

where

A_x: Peak area of the analyte

$C_{St,\ nominal}$: Nominal concentration of the analyte in external bracketing standard solutions, in µg/L

A_{St}: Peak area of the analyte in external standard solution (mean from bracketing standards)

df: Dilution factor: Dilution 1:2 for Tri-allate, df=2
No dilution for TCPSA, df=1

Recoveries were calculated by the following equation:

$$Rec = \frac{R_{found}}{R_{fortified}} \cdot 100\,\%$$

Rec: Recovery [%]

R_{found}: Analyte determined [µg/L]

$R_{fortified}$: Fortification level [µg/L]

Example calculation:

Recovery 0.1 µg/L in drinking water (Tri-allate, quantifier 304 → 86):

Sample ID: RDW 0.1-1

A_x: 28047 counts

$C_{St, nominal}$: 0.05 µg/L

A_{St}: 29420 counts (mean of two bracketing standards)

$$R = \frac{28047 \cdot 0.05\,\mu g/L}{29420} \cdot 2 = 0.0953\,\mu g/L$$

$$Rec = \frac{0.0953\,\mu g/L}{0.1\,\mu g/L} \cdot 100\% = 95\%$$

3.11 Standard Solutions

3.11.1 Stock Solutions

A stock solution containing 400 mg/L Tri-allate (S400 (10105)) was prepared as follows:

20.2 mg of the reference item (purity 98.8 %) were weighed into a 50 mL volumetric flask and the volume was adjusted to 50 mL with acetone.

Dilutions at 10 mg/L (S10 (10105)), 1 mg/L (S1 (10105)), 0.1 m/L (S0.1 (10105)) and 0.01 mg/L (S0.01 (10105)) were prepared in acetonitrile.

A stock solution containing 400 mg/L TCPSA (S400 (10106)) was prepared as follows:

20.1 mg of the reference item (purity 99.4 %) were weighed into a 50 mL volumetric flask and the volume was adjusted to 50 mL with methanol.

Dilutions at 10 mg/L (S10 (10106)), 1 mg/L (S1 (10106)), 0.1 m/L (S0.1 (10106)) and 0.01 mg/L (S0.01 (10106)) were prepared in methanol.

All solutions were stored at < 8°C in the dark.

3.11.2 Preparation of Standard Solutions for Tri-allate

Dilutions of the 1 mg/L standard solution of Tri-allate (S1(10105)) were prepared in acetonitrile. The preparation of these dilutions is described in Table 2. These dilutions were used for fortification of recovery samples and as pre-dilution step for preparation of matrix-matched calibration standards.

Table 2: Preparation of standard solutions of Tri-allate

Standard solution	Standard solution used for preparation	Volume used [µL]	Solvent used [µL]	Solvent	Concentration obtained [µg/L]
Std 500 µg/L	S1 (10105)	500	500	Acetonitrile	500
Std 250 µg/L	Std 500 µg/L	500	500		250
Std 100 µg/L	S1 (10105)	100	900		100
Std 50 µg/L	Std 100 µg/L	500	500		50
Std 25 µg/L	Std 50 µg/L	500	500		25
Std 10 µg/L	Std 100 µg/L	100	900		10
Std 5 µg/L	Std 50 µg/L	100	900		5
Std 2.5 µg/L	Std 5 µg/L	500	500		2.5

Std: Standard solution

3.11.3 Preparation of Matrix-matched Calibration Standards for the Determination of Tri-allate

For preparation of matrix-matched standard solutions, dilutions of Tri-allate standard solutions in acetonitrile (Table 2) were further diluted with drinking water as described in Table 3. These standard solutions (intermediate standard solutions) prepared in drinking water were finally diluted 1:2 with water (HPLC gradient grade) to receive matrix-matched calibration standards used for quantification of Tri-allate (Table 4).

Table 3: Preparation of intermediate standard solutions for preparation of matrix-matched standard solutions for Tri-allate

Standard solution	Standard solution used for preparation	Volume used [µL]	Final dilution volume* [mL]	Solvent	Concentration obtained [µg/L]
Std 5 µg/L V1	Std 500 µg/L	100	10	Drinking water	5
Std 2.5 µg/L V1	Std 250 µg/L	100	10		2.5
Std 1 µg/L V1	Std 100 µg/L	100	10		1
Std 0.5 µg/L V1	Std 50 µg/L	100	10		0.5
Std 0.25 µg/L V1	Std 25 µg/L	100	10		0.25
Std 0.1 µg/L V1	Std 10 µg/L	100	10		0.1
Std 0.05 µg/L V1	Std 5 µg/L	100	10		0.05
Std 0.025 µg/L V1	Std 2.5 µg/L	100	10		0.025

* using volumetric flasks; V1: Dilution 1 (intermediate standard solution)

Table 4: Preparation of matrix-matched calibration standard solutions for analysis of Tri-allate

Standard solution	Standard solution used for preparation	Volume used [µL]	Solvent used [µL]	Solvent	Concentration obtained [µg/L]
MStd 2.5 µg/L DW	Std 5 µg/L V1	500	500	Water (HPLC gradient grade)	2.5
MStd 1.25 µg/L DW	Std 2.5 µg/L V1	500	500		1.25
MStd 0.5 µg/L DW	Std 1 µg/L V1	500	500		0.5
MStd 0.25 µg/L DW	Std 0.5 µg/L V1	500	500		0.25
MStd 0.125 µg/L DW	Std 0.25 µg/L V1	500	500		0.125
MStd 0.05 µg/L DW	Std 0.1 µg/L V1	500	500		0.05
MStd 0.025 µg/L DW	Std 0.05 µg/L V1	500	500		0.025
MStd 0.0125 µg/L DW	Std 0.025 µg/L V1	500	500		0.0125

MStd: Matrix-matched standard solution; DW: Drinking water

In addition, standard solutions in pure solvent (HPLC gradient grade water) at LOQ and 10 fold LOQ were prepared accordingly using HPLC gradient grade water instead of drinking water to check for matrix effects.

3.11.4 Preparation of Standard Solutions for TCPSA

Dilutions of the 1 mg/L standard solution of TCPSA (S1(10106)) were prepared in methanol. The preparation of these dilutions is described in Table 5. These dilutions were used for fortification of recovery samples and as pre-dilution step for preparation of matrix-matched calibration standards.

Table 5: Preparation of standard solutions of TCPSA

Standard solution	Standard solution used for preparation	Volume used [µL]	Solvent used [µL]	Solvent	Concentration obtained [µg/L]
Std 250 µg/L M	S1 (10106)	100	300	Methanol	250
Std 100 µg/L M	S1 (10106)	100	900		100
Std 50 µg/L M	Std 100 µg/L M	500	500		50
Std 25 µg/L M	Std 50 µg/L M	500	500		25
Std 10 µg/L M	Std 100 µg/L M	100	900		10
Std 5 µg/L M	Std 50 µg/L M	100	900		5
Std 2.5 µg/L M	Std 5 µg/L M	50	500		2.5

Std: Standard solution; M: Metabolite (TCPSA)

3.11.5 Preparation of Matrix-matched Calibration Standards for the Determination of TCPSA

Matrix-matched calibration standards were prepared in drinking water by dilution of the TCPSA standard solutions prepared in methanol (Table 5). Preparation of these matrix-matched calibration standards is described in Table 6.

Table 6: Preparation of matrix-matched calibration standard solutions for analysis TCPSA

Standard solution	Standard solution used for preparation	Volume used [µL]	Solvent volume [mL]	Solvent	Concentration obtained [µg/L]
MStd 2.5 µg/L DWM	Std 250 µg/L M	50	5	Drinking water	2.5
MStd 1 µg/L DWM	Std 100 µg/L M	50	5		1
MStd 0.5 µg/L DWM	Std 50 µg/L M	50	5		0.5
MStd 0.25 µg/L DWM	Std 25 µg/L M	50	5		0.25
MStd 0.1 µg/L DWM	Std 10 µg/L M	50	5		0.1
MStd 0.05 µg/L DWM	Std 5 µg/L M	50	5		0.05
MStd 0.025 µg/L DWM	Std 2.5 µg/L M	50	5		0.025

MStd: Matrix-matched standard solution; DW: Drinking water; M: Metabolite (TCPSA)

4 Fortifications

Control (untreated) specimens were fortified prior to extraction with the standard solutions as follows:

Table 7: Fortification of specimens for matrix drinking water

Analyte	Specimen volume [mL]	Fortification level [µg/L]	Fortification solution	Added [µL]
Tri-allate	10	0.1	Std 10 µg/L	100
	10	1	Std 100 µg/L	100
TCPSA	5	0.1	Std 10 µg/L M	50
	5	1	Std 100 µg/L M	50

5 Deviations from the Study Plan

The study was performed according to the study plan dated 29 January 2018 with the following minor deviation. For characterisation of the water sample the TOC (Total Organic Carbon) instead of the DOC (Diluted Organic Carbon) has been analysed. Therefore, the TOC is provided in this final report.

This report reflects the conduct of the study.

6.2 Limit of Quantification, Limit of Detection, Blanks

The limit of quantification (LOQ) was defined as the lowest fortification level at 0.1 µg/L with mean recoveries ranging from 70 % to 110 % at a relative standard deviation (RSD) of ≤ 20 % and blanks not exceeding 30 % of the LOQ. These criteria were fulfilled for Tri-allate and TCPSA in matrix drinking water. The limit of detection (LOD) was defined as 30 % of the limit of quantification as required by SANCO/825/00 rev. 8.1 (2010) for residues in control samples (i.e. 0.03 µg/L). Residues in the untreated samples used for recovery experiments were below 30 % of the LOQ, respectively below the limit of detection (LOD).

6.3 Matrix Effects

The matrix effect was tested at two concentration levels corresponding to the LOQ and 10 fold LOQ level by injecting standard solutions in pure solvent against matrix-matched standard solutions (double injection). For both analytes matrix effects were found to be below 20 % in matrix drinking water. Nevertheless, matrix-matched calibrations were used.

6.4 Calibration Information

Tri-allate and TCPSA respectively, were used for preparing external calibration standard solutions in the range of 0.0125 µg/L to 2.5 µg/L (Tri-allate) and 0.025 µg/L to 2.5 µg/L (TCPSA). External standard calibrations (Tri-allate: eight-point calibration, TCPSA: seven-point calibaration) were carried out using the peak area in integrator units (counts) from injection of known standards versus standard concentrations in µg/L. In the analytical sequence after each 2-4 samples external standard solutions were injected. The found analyte concentration (Tri-allate and TCPSA, respectively), expressed in µg/L, was calculated against the bracketing standards to compensate drifting detector response.

6.5 Linearity

The linearity of the detector response was confirmed by injecting matrix-matched standard solutions covering the working range of 0.0125 µg/L to 2.5 µg/L (Tri-allate) and 0.025 µg/L to 2.5 µg/L (TCPSA) with correlation coefficients of r ≥ 0.9981 (Tri-allate) and r ≥ 0.9993 (TCPSA, two different columns). The lower margin of the linearity test was at least 30 % of the LOQ and the upper margin was higher by at least 20 % as the 10 fold LOQ. These margins cover the minimum range as required by SANCO/825/00 rev.8.1 (2010).

6.6 Selectivity

The concentrations of the analytes in prepared samples were determined by high performance liquid chromatography (HPLC) with MS/MS detection. For Tri-allate two mass transitions were monitored in order to ensure unambiguous identification. For TCPSA a single mass trasition was analysed using two chromatographic columns with different stationary phases (phenyl hexyl column and C18 column). No significant interferences from the specimen matrix were detected at the retention time corresponding to the analyte under investigation in any of the control specimens.

6.7 Stability

Analysis has been performed within 24 hours after sample preparation. The stability of final extracts during storage in a freezer (<-18°C) has been assessed for at least 7 days within the primary method validation study (López Benet, F., Laboratorio de Análisis de Residuos de Plaguicidas, Universitat Jaume I, study code 268-16, Spain, 2016).

7 Discussion and Conclusions

Analytical methods for analysis of residues of Tri-allate and TCPSA in drinking water, developed at Laboratorio de Análisis de Residuos de Plaguicidas, Universitat Jaume I, Castellón / Spain in 2016 (López Benet, F., study code 268-16), were successfully validated at a limit of quantification (LOQ) of 0.1 µg/L. The specimens were analysed using liquid chromatography with mass selective detection (HPLC-MS/MS).

The data presented demonstrate that the methods permit the determination of residues of Tri-allate and TCPSA in drinking water with satisfactory accuracy, precision and repeatability according to guideline SANCO/825/00 rev.8.1 (2010).