STUDY TITLE

Validation of analytical methodology for the determination of residues of Triallate and its metabolite TCPSA in water (drinking, ground and surface waters)

GUIDELINES

SANCO/3029/99 rev. 4, SANCO/825/00 rev. 8.1, OECD 39
(ENV/JM/MONO(2007)17 and EPA OPPTS 860.1340)

1. Introduction

The objective of this Study was to develop and validate analytical methodology, based on liquid chromatography coupled to mass spectrometry in tandem (LC-MS/MS), for the determination of Tri-allate and TCPSA (as 2,3,3-trichloropropen-2-sulfonic acid sodium salt) residues in different water samples (drinking, ground and surface waters).

This Report describes the analytical methods developed, detailing their validation which has been carried out using blank samples fortified with Tri-allate and blank samples fortified with TCPSA.

2. Instrumentation and Apparatus

Following instrumentation and apparatus has been used in the study:

- ✓ Analytical balances Mettler AE200 and Mettler AB265S (Mettler, Barcelona, Spain)
- ✓ Centrifuge Consul (Orto-Alresa, Ajalvir, Madrid, Spain)
- ✓ LC-MS/MS Xevo TQD (Waters, Milford, MA, USA)
- ✓ LC-MS/MS Xevo TQS (Waters, Milford, MA, USA)

3. Materials and Reagents

3.1. Reference item

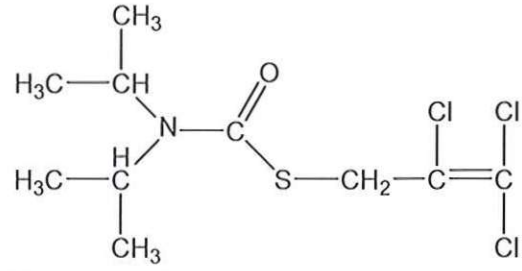
The target compounds of this study are Tri-allate and TCPSA. Due to the characteristics of this validation study, there is not any application of test items in field.

Reference items (analytical standards of Tri-allate and TCPSA) were received and stored according to the LARP SOP A014/6.

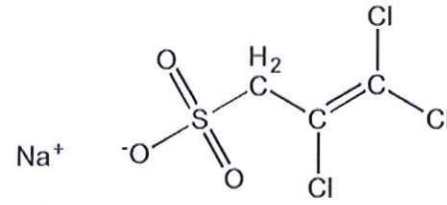
Certificates of analysis of the reference items are included in the Appendix 1 of this Final Report.

The main characteristics of the reference items are shown below.

Tri-allate

Chemical name:	S-(2,3,3-trichloroprop-2-en-1-yl) di(propan-2-yl) carbamothioate
Supplier	Sigma-Aldrich
CAS Number:	2303-17-5
Molecular formula:	$C_{10}H_{16}Cl_3NOS$
Molecular weight:	304.66 g/mol
Structural formula:	
Purity	98.8%
Appearance / colour	Solid colourless
Certificate of analysis	12/11/2013
Expiry date	28/10/2018
Lot number	SZBD301XV
Storage requirements	2-8°C
Date of receipt	14/12/2015
Quantity	0.25 g

TCPSA

Chemical name:	2,3,3-trichloropropen-2-sulfonic acid sodium salt
Supplier	SYNPURA
CAS Number:	65600-61-5
Molecular formula:	$C_3H_2Cl_3NaO_3S$
Molecular weight:	247.46 g/mol
Structural formula:	
Purity	99.8%
Appearance / colour	Colourless solid
Certificate of analysis	06/10/2015
Expiry date	06/10/2016
Lot number	SP15-106-1-1
Storage requirements	Room temperature
Date of receipt	22/12/2015
Quantity (g)	1

Full-scan and MS/MS spectra for Tri-allate were obtained in a previous study in the LARP: "Validation of analytical methodology for the determination of residues of Tri-allate and TCPSA in vegetal samples" (code 261-15, study director Francisco Lopez).

Positive electrospray full-scan and MS/MS spectra obtained from injection of a standard solution of Tri-allate of approx. 0.5 mg/L in methanol/water (50:50. v/v) containing 0.1% formic acid are shown in Figure 1. As it can be seen in Figure 1, Tri-allate shows an abundant $[M+H]^+$ peak at *m/z* 304, which has been selected as precursor ion. According to the fragmentation of this ion, we have selected the acquisition of mass transitions *m/z*=304 > *m/z*=86 (quantification) and *m/z*=304 > *m/z*=128 (confirmation).

Full-scan and MS/MS spectra for TCPSA were obtained in a previous study in the LARP: "Validation of analytical methodology for the determination of residues of Tri-allate and its metabolite TCPSA in soil samples" (code 266-16, study director Francisco Lopez).

A standard solution of TCPSA of 53 µg/L (water/methanol, 90:10, v/v) was injected on the LC-MS/MS Xevo TQS instrument in full scan mode, optimizing and obtaining the combined spectra for the chromatographic peak of TCPSA (Figure 2, top). As it can be seen, TCPSA shows an abundant $[M+H]^-$ peak at *m/z* 223, which has been selected as precursor ion. The fragmentation of this ion (Figure 2, bottom), working in daughter scan mode, makes possible the acquisition of two selective SRM transitions: *m/z*=223 > *m/z*=80 and *m/z*=223 > *m/z*=187. However, the validation requirements cannot be satisfied with the transition *m/z*=223 > *m/z*=187 due to an interference from matrix. Therefore, as only one transition is acquired, two injections in different chromatographic columns (phenyl hexyl and C18) were required for confirmation purposes.

A stock solution of TCPSA of 532 mg/L (ref. TCPSA/01) was prepared by dissolving 53.35 mg of the standard (lot number SP15-106-1-1, purity 99.8%) in methanol (100 mL).

Preparation of standard solutions

Several standard solutions of Tri-allate were prepared from the 495 µg/mL standard solution of Tri-allate in acetone by dilution with acetonitrile using glass pipettes and volumetric flasks according to the LARP SOPs C012/9. Next table shows an example of preparation of these standards solutions.

Table 1. ***Preparation of standards solutions of Tri-allate***

Initial concentration	Prepared by removal of (mL)	Dilution to (mL)	Final concentration
495 mg/L	2.5	25	49.5 mg/L
49.5 mg/L	2.5	25	4.95 mg/L
4.95 mg/L	5	10	2.48 mg/L
4.95 mg/L	2.5	25	495 µg/L
2.48 mg/L	2.5	25	248 µg/L
495 µg/L	5	25	99.0 µg/L
495 µg/L	2.5	25	49.5 µg/L
248 µg/L	2.5	25	24.8 µg/L
99.0 µg/L	2.5	25	9.90 µg/L
49.5 µg/L	2.5	25	4.95 µg/L
24.8 µg/L	2.5	25	2.48 µg/L

Several standard solutions of TCPSA were prepared from the 532 mg/L standard solution of TCPSA in methanol by dilution with methanol using glass pipettes and volumetric flasks according to the LARP SOPs C012/9. Next table shows an example of preparation of these standards solutions.

Table 2. ***Preparation of standards solutions of TCPSA***

Initial concentration	Prepared by removal of (mL)	Dilution to (mL)	Final concentration
532 mg/L	5	25	106 mg/L
106 mg/L	5	20	26.5 mg/L
106 mg/L	2.5	25	10.6 mg/L
26.5 mg/L	5	25	5.30 mg/L
26.5 mg/L	2.5	25	2.65 mg/L
10.6 mg/L	2.5	25	1.06 mg/L
5.30 mg/L	2.5	25	530 µg/L
2.65 mg/L	2.5	25	265 µg/L
1.06 mg/L	2.5	25	106 µg/L
530 µg/L	2.5	25	53.0 µg/L
265 µg/L	2.5	25	26.5 µg/L
106 µg/L	2.5	25	10.6 µg/L
53.0 µg/L	2.5	25	5.30 µg/L
26.5 µg/L	2.5	25	2.65 µg/L

Fortification solutions

Standard solution of Tri-allate of 9.90 µg/L was used as fortification solution for LOQ level whereas standard solution of 99.0 µg/L was used for 10 × LOQ level.

Standard solution of TCPSA of 10.6 µg/L was used as fortification solution for LOQ level whereas standard solutions of 106 µg/L was used for 10 × LOQ level.

Stability of standards solutions

Stability studies of standard solutions of Tri-allate in acetonitrile and TCPSA in methanol stored at different times, both in refrigerator (at temperatures between 0.5 and 7ºC) and in freezer (at temperatures below -18ºC), were carried out according to LARP SOP C012/9 in two previous studies in the LARP: "Validation of analytical methodology for the determination of residues of Tri-allate in vegetal samples" (Study code 253-11; Study Director, Francisco Lopez) and "Validation of analytical methodology for the determination of residues of TCPSA in vegetal samples" (Study code 254-11; Study Director, Francisco López).

Results obtained proved the stability of standards solutions in all cases during the maximum time tested: 2 months in freezer and 1 month in refrigerator.

3.3. Reagents

- Acetone residue analysis quality (Scharlab, Barcelona, Spain)
- Acetonitrile HPLC quality (Scharlab, Barcelona, Spain)
- Formic acid 98-100%, reagent grade (Scharlab, Barcelona, Spain)
- Methanol HPLC quality (Scharlab, Barcelona, Spain)
- Water: HPLC quality obtained by purifying deionized water in a Milli-Q Gradient A10 system (Millipore, Molsheim, Germany)

4. Specimens

The validation of the developed analytical methodology in the different water samples was carried out using blank samples, which were sampled by the LARP personnel. All samples were received in the LARP on 04/05/2016 according to the LARP SOP A013/9.

Next table shows the different samples obtained, including information about their origin.

Table 3. ***List of water samples***

Type of water	LARP Code	Origin
Drinking water	VAL268/1	Public water supply (Grao de Castellón, Spain)
	VAL268/2	Public water supply (Castellón, Spain)
Surface water	VAL268/3	Clot de la Mare de Deu (Burriana, Spain)
	VAL268/4	Millars river (Castellón, Spain)
Groundwater	VAL268/5	Well located in Castellon (Spain)
	VAL268/6	Well located in Burriana (Spain)

During reception, each sample (ca. 5 L) was divided in different subsamples which were stored frozen (Table 4).

Table 4. ***List of subsamples***

LARP Code	Subsamples
VAL268/1	VAL268/1/A, VAL268/1/B, VAL268/1/C, VAL268/1/D, VAL268/1/E, VAL268/1/F, VAL268/1/G
VAL268/2	VAL268/2/A, VAL268/2/B, VAL268/2/C, VAL268/2/D
VAL268/3	VAL268/3/A, VAL268/3/B, VAL268/3/C, VAL268/3/D
VAL268/4	VAL268/4/A, VAL268/4/B, VAL268/4/C, VAL268/4/D, VAL268/4/E, VAL268/4/F, VAL268/4/G
VAL268/5	VAL268/5/A, VAL268/5/B, VAL268/5/C, VAL268/5/D, VAL268/5/E, VAL268/5/F, VAL268/5/G
VAL268/6	VAL268/6/A, VAL268/6/B, VAL268/6/C, VAL268/6/D

Subsamples B (ca. 0.5 L) were sent to the laboratory IPROMA to be characterized according to ISO/IEC 17025. These analyses do not have carried out in compliance with Good Laboratory Practices. Results obtained are shown in the next table.

Table 5. ***Chemical characteristics of water samples***

Sample	DOC (mg/L)	pH	Conductivity at 20°C (µS/cm)	Residues of evaporation at 180°C (mg/L)	Hardness (mg/L)
VAL268/1	< 1.0	7.2	104	48	8
VAL268/2	< 1.0	7.9	303	182	136
VAL268/3	2.0	8.5	1456	1069	684
VAL268/4	< 1.0	8.4	1730	1111	548
VAL268/5	< 1.0	8.5	1356	1089	700
VAL268/6	1.2	8.4	1554	1299	834

*French degree; °f = 10 mg/L $CaCO_3$

Analysis certificates of water samples are included in Appendix 2 of this Final Report.

5. Analytical methodology for Tri-allate

An analytical method based on LC-MS/MS for the determination of Tri-allate in water samples has been validated (Standard Operation Procedure from the LARP SOP E168/1). This procedure is described in detail in the following sections. Instrumental methods are included in the Appendix 3 of this Final Report.

5.1. Sample preparation

0.5 mL of water sample and 0.5 mL of HPLC water were transferred into an autosampler vial.

5.2. LC-MS/MS determination

The sample analysis is carried out using the following conditions for the LC-MS/MS system (LARP SOP C047/3):

Column: Kinetex XB-C_{18} 5 µm, 50 x 2.1 mm, part number 00B-4605-AN (Phenomenex, Torrance, CA, USA).

Mobile phase:

- Solvent A: HPLC water containing 0.01% formic acid
- Solvent B: HPLC methanol containing 0.01% formic acid

Table 6. ***Mobile phase gradient for the determination of Tri-allate***

time (min)	% A	% B
0.00	60	40
1.00	60	40
4.00	5	95
8.00	5	95
8.10	60	40

Column temperature: room temperature

Flow rate: 0.200 mL/min

Chromatographic analysis time: 12 min

Solvent delay: 0-6 min and 8-12 min

Injection volume: 15 µL

Interface: electrospray (positive ionization mode)

Capillary voltage: 3.5 kV

Extractor: 3 V

Source temperature: 150ºC

Desolvation temperature: 650ºC

Desolvation gas: dry nitrogen, about 1200 L/h

Collision gas: argon C-45, about 3.5 x 10^{-3} mbar

Cone gas: 60 L/h

Retention time: 6.9 min

Table 7. *MS/MS conditions for the determination of Tri-allate*

precursor ion (m/z)	*dwell* time (s)	cone (V)	collision energy (eV)	product ion (m/z)	
304	0.080	40	15	86	Q
304	0.080	40	15	128	q

Q: quantitative transition. q: confirmative transition

5.3. Calibration curves preparation

Quantification of Tri-allate is carried out by means of external calibration using standard solutions prepared in HPLC water. 0.1 mL of standard solutions of Tri-allate in acetonitrile were diluted with HPLC water to a final volume of 10 mL. Then, 0.5 mL of these standards and 0.5 mL of HPLC water were introduced in an autosampler vial.

Table 8. *Preparation of calibration standard solutions of Tri-allatte*

Concentration of acetonitrile standards (µg/L)	Concentration of HPLC water standards[a] (µg/L)	Concentration of calibration solutions[b] (µg/L)
2.48	0.025	0.012
4.95	0.050	0.025
9.90	0.099	0.050
24.8	0.248	0.124
49.5	0.495	0.248
99.0	0.990	0.495
248	2.48	1.24
495	4.95	2.48

[a] Obtained by dilution of 0.1 mL of acetonitrile standards to a final volume of 10 mL with HPLC water

[b] Obtained by dilution of 0.5 mL of HPLC water standards with 0.5 mL of HPLC water

5.4. Fortification procedure

Fortification procedure of blank samples is carried out as follows: 100 µL of a standard solution of Tri-allate in acetonitrile at concentrations of 9.90 µg/L (for 0.1 µg/L nominal fortification level) or 99.0 µg/L (for 1 µg/L nominal fortification level) were added to a 10 mL volumetric flask. Then, the volume was adjusted to 10 mL with blank water sample.

5.5. Calculation of residues

Tri-allate residues in samples were calculated according to the following equation

$$R = C \times d$$

R residue (µg/L)
C Tri-allate concentration in final extract, as calculated from the peak area, obtained from the calibration curve in µg/L
d dilution factor = 2

Recoveries were calculated by the following equation

$$Rec = \frac{R_{found}}{R_{fortified}} \times 100$$

Rec recovery (%)
R_{found} residue determined (µg/L)
$R_{fortified}$ fortification level (µg/L)

An example of calculation is included in the Appendix 4 of this Final Report.

6. Analytical methodology for TCPSA

An analytical method based on LC-MS/MS for the determination of TCPSA in water samples has been validated (Standard Operation Procedure from the LARP SOP E170/1). This procedure is described in detail in the following sections. Instrumental methods are included in the Appendix 3 of this Final Report.

6.1. Sample preparation

An aliquot of water sample, previously centrifuged if presented turbidity, was filtered through a 0.2 µm PTFE filter and transferred into an autosampler vial.

6.2. LC-MS/MS determination

Samples are injected on both next columns:

- ✓ Kinetex Phenyl Hexyl 5 µm, 50 x 2.1 mm, part number 00B-4603-AN (Phenomenex, Torrance, CA, USA)
- ✓ Kinetex C18 5 µm, 50 x 2.1 mm, part number 00B-4601-AN (Phenomenex, Torrance, CA, USA)

The injection on Phenyl Hexyl column is used for quantification, whereas injection on C18 column is used as confirmative purposes.

The sample analysis is carried out using the following conditions for the LC-MS/MS system (LARP SOP C048/1):

Mobile phase:

- Solvent A: HPLC water containing 0.01% formic acid
- Solvent B: HPLC acetonitrile

Table 9. ***Mobile phase gradient for the determination of TCPSA***

time (min)	% A	% B
0.00	90	10
6.00	10	90
9.00	10	90
9.10	90	10
15.0	90	10

Column temperature: room temperature

Flow rate: 0.200 mL/min

Chromatographic analysis time: 15 min

Solvent delay: 6-15 min

Injection volume: 25 µL

Interface: electrospray (negative ionization mode)

Capillary voltage: 2.5 kV

Source temperature: 150ºC

Desolvation temperature: 650ºC

Desolvation gas: dry nitrogen, about 1200 L/h

Collision gas: argon C-45, about 3.5×10^{-3} mbar

Cone gas: 250 L/h

Retention time on Phenyl Hexyl column: 3.3-3.4 min

Retention time on C18 column: 1.9-2.0 min

Table 10. ***MS/MS conditions for the determination of TCPSA***

precursor ion (m/z)	*dwell* time (s)	cone (V)	collision energy (eV)	product ion (m/z)
223	0.080	45	20	80

6.3. Calibration curves preparation

Quantification of TCPSA was carried out by using matrix-matched calibration. Standards were prepared by adding 50 µL of standard solutions in methanol to 5.0 mL of blank extract. Then, calibration standards were filtered through a 0.2 µm PTFE filter.

Table 11. ***Preparation of matrix-matched calibration standards solutions of TCPSA***

Initial concentration (µg/L)	Volume taken (µL)	Blank extract volume (µL)	Final Concentration (µg/L)
2.65	50	5000	0.026
5.30	50	5000	0.052
10.6	50	5000	0.105
26.5	50	5000	0.262
53.0	50	5000	0.525
106	50	5000	1.05
265	50	5000	2.62

6.4. Fortification procedure

Fortification procedure of blank samples is carried out as follows: 50 µL of a standard solution of TCPSA in methanol at concentration of 10.6 µg/L or 106 µg/L were added to 5 mL of blank sample for 0.1 µg/L and 1 µg/L nominal fortification level, respectively.

6.5. Calculation of residues

TCPSA residues in samples were calculated according to the following equation

$$R = C$$

R residue (µg/L)
C TCPSA concentration in final extract, as calculated from the peak area, obtained from the calibration curve in µg/L

Recoveries were calculated by the following equation

$$Rec = \frac{R_{found}}{R_{fortified}} \times 100$$

Rec recovery (%)
R_{found} residue determined (µg/L)
$R_{fortified}$ fortification level (µg/L)

An example of calculation is included in the Appendix 4 of this Final Report.

7. Validation results for Tri-allate

The analytical method for the determination of Tri-allate in water samples was validated using blank samples. All parameters have been calculated for both SRM transitions.

7.1. Linearity

Linearity of the method was studied in the range of nominal concentrations between 0.01 and 2.5 µg/L of Tri-allate (corresponding to 0.02-5 µg/L Tri-allate in water), including eight standard solutions.

Three complete calibration sets were injected in one sequence. Within each calibration set, standards from the lowest to the highest concentration were measured. Linear calibration was applied.

7.2. Accuracy and Precision

Accuracy of the analytical method was studied by means of recovery experiments with blank water samples fortified at two concentration levels (0.1 and 1 µg/L) with Tri-allate (5 replicates). Samples fortified at each concentration level were injected between two calibration sets (injected from the lowest concentration until the highest). The same vial was used for each level of concentration and average response from the previous and subsequent calibration was used for quantification purposes.

Precision was also estimated from these experiments.

Drinking water

Fortified drinking water samples were prepared (from blank sample identified as VAL268/1/C), extracted and analyzed on 11/05/2016.

Drinking water

A LOQ of 0.1 µg/L was established for Tri-allate in drinking water samples for both transitions (*m/z*=304 > *m/z*=86; *m/z*=304 > *m/z*=128).

Surface water

A LOQ of 0.1 µg/L was established for Tri-allate in surface water samples for both transitions (*m/z*=304 > *m/z*=86; *m/z*=304 > *m/z*=128).

Groundwater

A LOQ of 0.1 µg/L was established for Tri-allate in groundwater samples for both transitions (*m/z*=304 > *m/z*=86; *m/z*=304 > *m/z*=128).

7.4. Limit of Detection (LOD)

LOD values were calculated from chromatograms corresponding to blank water samples fortified with Tri-allate at a nominal concentration of 0.1 µg/L. The signal/noise ratio (S/N) for the Tri-allate peak was calculated using the chromatographic software. The LOD was defined as the concentration corresponding to a chromatographic peak with a signal/noise ratio of 3.

Drinking water

The LOD for Tri-allate in drinking water samples was estimated to be 0.02 µg/L for transition *m/z*=304 > *m/z*=86 and 0.01 µg/L for transition *m/z*=304 > *m/z*=128.

Surface water

The LOD for Tri-allate in surface water samples was estimated to be 0.01 µg/L for transition *m/z*=304 > *m/z*=86 and 0.02 µg/L for transition *m/z*=304 > *m/z*=128.

Groundwater

The LOD for Tri-allate in groundwater samples was estimated to be 0.01 µg/L for transition *m/z*=304 > *m/z*=86 and 0.02 µg/L for transition *m/z*=304 > *m/z*=128.

Consequently, a LOD of 0.02 µg/L for Tri-allate was established for all type of waters.

7.5. Specificity

Specificity of the method was studied by injecting for each type of water, a solvent blank obtained by applying the extraction procedure without sample, two blank samples and a blank sample fortified with Tri-allate at a nominal concentration of 0.1 µg/L. The acceptance criterion established in the Study Plan states that signal of the blanks must be lower than 30% of LOQ signal.

Drinking water

No response was obtained for Tri-allate in the blanks. LC-MS/MS chromatograms for Tri-allate corresponding to the solvent blank 268/BP/1 (Figure 29), blank water samples VAL268/1/C (Figure 30) and VAL268/2/C (Figure 31), and the blank water sample fortified 268/F0.1/1 (Figure 32) are included in the Appendix 6 of this Report.

Surface water

No response was obtained for Tri-allate in the blanks. LC-MS/MS chromatograms for Tri-allate corresponding to the solvent blank 268/BP/2 (Figure 33), blank water samples VAL268/3/C (Figure 34) and VAL268/4/C (Figure 35), and the blank water sample fortified 268/F0.1/6 (Figure 36) are included in the Appendix 6 of this Report.

Groundwater

No response was obtained for Tri-allate in the blanks. LC-MS/MS chromatograms for Tri-allate corresponding to the solvent blank 268/BP/3 (Figure 37), blank water samples VAL268/5/C (Figure 38) and VAL268/6/C (Figure 39), and the blank water sample fortified 268/F0.1/11 (Figure 40) are included in the Appendix 6 of this Report.

7.6. Stability of extract solutions

Stability of extract solutions was studied by re-analysing extracts from blank water samples fortified with Tri-allate at 1 µg/L, which were stored in the freezer (at temperatures below -18ºC) during at least 7 days. Both aliquots of the raw extracts (before dilution with HPLC water) and second vials with final extract (after dilution) were stored in the freezer and analysed again.

No significant matrix effects (difference below 20%) were observed, both comparing slope of the calibration curves and responses for standards (individual values and average). Then, quantification of Tri-allate in groundwater samples can be carried out by external calibration using standard solutions prepared in HPLC water.

Figures 79-82 of Appendix 7 show the calibration curves and the plots response factor versus concentration corresponding to the study of matrix effects in the analysis of Tri-allate in groundwater samples for quantification and confirmation transitions.

8. Validation results for TCPSA

The analytical method for the determination of TCPSA in water samples was validated using blank samples. All parameters have been calculated for both injections. Results are shown below.

8.1. Linearity

Linearity of the method was studied in the range of nominal concentrations between 0.025 and 2.5 µg/L of TCPSA (corresponding to 0.025-2.5 µg/L TCPSA in water), including seven standard solutions. Three complete calibration sets were injected in one sequence. Within each calibration set, standards from the lowest to the highest concentration were measured. Linear calibration was applied. The next tables show the results obtained.

Drinking water

Matrix matched calibration standards were prepared using blank drinking water sample VAL268/1/A.

Drinking water

A LOQ of 0.1 µg/L was established for TCPSA in drinking water samples for both injections.

Surface water

A LOQ of 0.1 µg/L was established for TCPSA in surface water samples for both injections.

Groundwater

A LOQ of 0.1 µg/L was established for TCPSA in groundwater samples for both injections.

8.4. Limit of Detection (LOD)

LOD values were calculated from chromatograms corresponding to a calibration standard in matrix of a concentration of 0.026 ng/mL. The signal/noise ratio (S/N) for the TCPSA peak was calculated using the chromatographic software. The LOD was defined as the concentration corresponding to a chromatographic peak with a signal/noise ratio of 3.

Drinking water

The LOD for TCPSA in drinking water samples was estimated to be 0.005 µg/L for quantification injection and 0.004 µg/L for confirmation injection.

Surface water

The LOD for TCPSA in surface water samples was estimated to be 0.004 µg/L for quantification injection and 0.006 µg/L for confirmation injection.

Groundwater

The LOD for TCPSA in groundwater samples was estimated to be 0.004 µg/L for quantification injection and 0.006 µg/L for confirmation injection.

Consequently, a LOD of 0.005 µg/L for TCPSA was established for drinking water samples, whereas for surface and groundwater samples a LOD of 0.006 µg/L TCPSA was established.

8.5. Specificity

Specificity of the method was studied by injecting for each type of water, a solvent blank obtained by applying the extraction procedure without sample, two blank samples and a blank sample fortified with TCPSA at a nominal concentration of 0.1 µg/L. The acceptance criterion established in the Study Plan states that signal of the blanks must be lower than 30% of LOQ signal.

Drinking water

No response was obtained for TCPSA in the blanks. LC-MS/MS chromatograms for TCPSA corresponding to the solvent blank 268/BP/5 (Figure 59), blank water samples VAL268/1/A (Figure 60) and VAL268/2/A (Figure 61), and the blank water sample fortified 268/F0.1/31 (Figure 62) are included in the Appendix 6 of this Report.

Surface water

No response was obtained for TCPSA in the blanks. LC-MS/MS chromatograms for TCPSA corresponding to the solvent blank 268/BP/6 (Figure 63), blank water samples VAL268/3/A (Figure 64) and VAL268/4/A (Figure 65), and the blank water sample fortified 268/F0.1/36 (Figure 66) are included in the Appendix 6 of this Report.

Groundwater

No response was obtained for TCPSA in the blanks. LC-MS/MS chromatograms for TCPSA corresponding to the solvent blank 268/BP/7 (Figure 67), blank water samples VAL268/5/A (Figure 68) and VAL268/6/A (Figure 69), and the blank water sample fortified 268/F0.1/41 (Figure 70) are included in the Appendix 6 of this Report.

8.6. Stability of extract solutions

Stability of extract solutions was studied by re-analysing extracts from blank water samples fortified with TCPSA at 1 µg/L, which were stored in the freezer (at temperatures below -18ºC) during at least 7 days. Next tables show the concentrations and recoveries obtained for each replicate at initial and final time, as well as the deviation between initial and final concentration expressed in percentage (absolute value).

Appendix 4. Examples of calculation of residues

Example of calculation of Tri-allate residues

Response (S) for sample 268/F0.1/1: 221.268

Calibration curve equation: $S = -8.43 + 4955.17 \times C$ (µg/L) (r = 0.9984)

$$C = \frac{221.268 + 8.43}{4955.17} = 0.046 \text{ µg/L}$$

$$R = C \times d = 0.046 \times 2 = 0.093 \text{ µg/L}$$

$R_{fortified}$ (sample 268/F0.1/1) = 0.099 µg/L

$$Rec = \frac{R_{found}}{R_{fortified}} \times 100 = \frac{0.093}{0.099} \times 100 = 93.6\%$$

Example of calculation of TCPSA residues

Response (S) for sample 268/F0.1/36: 3245.885

Calibration curve equation: $S = -79.08 + 32140.85 \times C$ (µg/L) (r = 0.9998)

$$C = \frac{3245.885 + 79.80}{32140.85} = 0.103 \text{ µg/L}$$

$$R = C = 0.103 \text{ µg/L}$$

$R_{fortified}$ (sample 268/F0.1/36) = 0.105 µg/L

$$Rec = \frac{R_{found}}{R_{fortified}} \times 100 = \frac{0.103}{0.105} \times 100 = 98.6\%$$