REGISTRATION REPORT

Part B

Section 5

Analytical Methods

Detailed summary of the risk assessment

Product code: SAP2101F

Product name(s): ZELORA START

Chemical active substance:

Prothioconazole, 120 g/L

Folpet, 300 g/L

Central Zone

Zonal Rapporteur Member State: Poland

CORE ASSESSMENT

(authorization)

Applicant: Selectis Produtos para a Agricultura, S.A.

Submission date: December 2023

Updated: April 2024, May 2024

MS Finalisation date: June 2024 (initial Core Assessment)

August 2024 (final Core Assessment)

Version history

When	What
December 2023	V0 - Initial version submitted by the Selectis Produtos para a Agricultura, S.A. for submission to Poland in the frame of new PPP registration (Acording Art. 33 of Regulation EC No 1107/2009)
April 2024	V1 – Updated version from Aplicant after receiving a request from the authorities. Updated are highlighted in yellow
May 2024	Updated version based on folpet data provided by the Selectis Produtos para a Agricultura, S.A. answering Poland comments in the frame of SAP50SCF/Folpec registration
June 2024	Initial zRMS assessment The report in the dRR format has been prepared by the Applicant, therefore all comments, additional evaluations and conclusions of the zRMS are presented in grey commenting boxes. Minor changes are introduced directly in the text and highlighted in grey. Not agreed or not relevant information are ~~struck through~~ and shaded for transparency. Following the evaluation and before sending the document for commenting, all coloured highlighting was removed from the parts updated by the Applicant, and all the text fragments struck through by the applicant as the result of the updates have been removed completely from the document, for better legibility.
August 2024	Final report (Core Assessment updated following the commenting period) No additional information or assessments after the commenting period.

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5 Analytical methods

5.1 Conclusion and summary of assessment

Sufficiently sensitive and selective analytical methods are available for the active substance(s) and relevant impurities in the plant protection product.
Noticed data gaps are:
None.

Sufficiently sensitive and selective analytical methods are available for all analytes included in the residue definitions.
Noticed data gaps are:

Folpet:

- lower LOQ at 0.01 mg/L for phthalimide for body fluids and should be provided at the renewal of the active substance and/or re-evaluation of plant production product.

Prothioconazole:

- analytical methods with appropriate ILVs for the determination of prothioconazole in all major matrix groups with an LOQ of 0.01 mg/kg is required and should be provided as a post-registration requirement;
- analytical methods with ILVs with appropriate LOQ for the determination of prothioconazole in animal matrices is required and should be provided as a post-registration requirement;
- an independent laboratory validation (ILV) for the method for the determination of prothioconazole residues in drinking water is required and should be provided at the renewal of the active substance and/or re-evaluation of plant production product;
- an analytical method for the residues of prothioconazole in body fluids and tissues is required and should be provided at the renewal of the active substance and/or re-evaluation of plant production product.

Commodity/crop	Supported/Not supported
Dry commodities / Wheat	Supported
Dry commodities / Barley	Supported

5.2 Methods used for the generation of pre-authorization data (KCP 5.1)

5.2.1 Analysis of the plant protection product (KCP 5.1.1)

5.2.1.1 Determination of active substance and/or variant in the plant protection product (KCP 5.1.1)

An overview on the acceptable methods and possible data gaps for analysis of prothioconazole and folpet in plant protection product is provided as follows:

Comments of zRMS:	Study acceptable. The analytical method for the determination of folpet and prothioconazole in ZELORA START was fully validated according to SANCO/3030/99 rev. 5.

Reference:	KCP 5.1.1/01
Report	PROTHIOCONAZOLE 120 g/L + FOLPET 300 g/L SC (SAP2101F): Physical, chemical and technical properties of the plant protection product, Morais, F., 2022, Report no EF/371/21 – Interim Report (T0) – Annex 1.
Guideline(s):	Yes, SANCO/3030/99 rev. 5
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods
The sample is dissolved in acetonitrile, the solution is placed in an ultra-sonic bath and filtered. Prothioconazole and folpet content are quantified using a UPLC-PDA method.

Chromatographic conditions – UPLC-PDA for active substances quantification

Mobile phase	Acetonitrile: 0.25% ammonium acetate (50:50)	
Run time	5 min	
Flow	0.250 mL/min	
Column	Acquity C18 UPLC BEH; 50 mm × 2.1 mm; 1.7 µm	
Column temperature	30 ºC	
Sample temperature	10 ºC	
Detection wavelength	225 nm	
Injection volume	0.3 µL	
Retention time	Prothioconazole:	Around 1.8 minutes
	Folpet:	Around 2.4 minutes

Chromatographic conditions – UPLC-MS/MS for prothioconazole identification

Run time	5 min
MS Ionization mode	ES+
Type	MS2 Scan
Data format	Centroid
Mass range	50 *m/z* – 500 *m/z*

Chromatographic conditions – GLC-MS/MS for folpet identification

Injector method	*Injection volume*	1.00 µL
	Pre-inj dwell time	3 s
	Post-inj dwell time	3 s
GC method	*Temperature*	80 ºC 15.0 ºC/min until 250 ºC (maintain for 8.67 minutes)

	Column	TG-5MS, 30 m × 0.25 mm × 0.25 µm film thickness
	S/SL mode	Split
	Inlet temperature	225 ºC
	Split flow	30.0 mL/min (constant flow)
	Carrier flow	1.500 mL/min
Detector Method (MS/MS)	*Temperature*	280 ºC
	Ion source	230 ºC
	Start time	2.0 minutes
	Ionization mode	EI
	Ion polarity	Positive
	Acquisition mode	SCAN (m/z 100 – 600 amu)
Retention times	*Folpet*	Around 11.5 minutes

Standard solution preparation

Prothioconazole: weigh, in duplicate, about 50 mg ± 10% of prothioconazole reference material into a 25 mL volumetric flask. Dissolve and complete the volume with acetonitrile (2.0 mg/mL).

Folpet: weigh, in duplicate, about 50 mg ± 10% of folpet reference material into a 25 mL volumetric flask. Dissolve and complete the volume with acetonitrile (2.0 mg/mL).

Final solution: prepare a calibration solution diluting 0.5 mL of prothioconazole standard stock solution and 1.2 mL of folpet standard stock solution to a final volume of 10 mL completing the volume with acetonitrile (0.10 $mg_{prothioconazole}$/mL and 0.24 mg_{folpet}/mL). (Solutions STD1 and STD2).

Sample solution preparation

Weigh approximately 94.4 mg ± 10% of sample into a 100 mL volumetric flask. Dissolve and complete the volume with acetonitrile. Place the solution in an ultra-sonic bath, filter using a 0.20 µm disk filter (0.94 mg/mL). Prepare in duplicate.

Validation - Results and discussions

Table 5.2-1: Methods suitable for the determination of active substance folpet in plant protection product SAP2101F

	Prothioconazole	**Folpet**
Author(s), year	Morais, F., 2022	Morais, F., 2022
Principle of method	UPLC-PDA	UPLC-PDA
Linearity (linear between mg/L / % range of the declared content) (correlation coefficient, expressed as r)	Range: [3.17% - 52.91%], [0.0299 mg/mL – 0.4995 mg/mL] y = 28909.0560x + 29.8700 r = 1.0000	Range: [4.22% - 105.72%], [0.0398 mg/mL – 0.9980 mg/mL] y = 185378.2614x + 3844.3943 r = 0.9985
Precision – Repeatability Mean n = 5 (%RSD)	System repeatability: RSD = 0.51%, Hr = 0.28 Method repeatability: RSD = 1.35%, Hr = 0.73 (expected content: 12% w/w, RSD criterion < 1.84%)	System repeatability: RSD = 0.46%, Hr = 0.29 Method repeatability: RSD = 0.51%, Hr = 0.32 (expected content: 30% w/w, RSD criterion < 1.61%)
Accuracy n = 5 (% Recovery) Total recovery	1st level (5.30%): 100.19% (RSD = 1.48%) (RSD criterion < 2.09%) 2nd level (10.59%): 100.02% (RSD = 0.86%) (RSD criterion < 1.88%)	1st level (6.36%): 99.85% (RSD = 0.46%) (RSD criterion < 2.03%) 2nd level (25.42%): 99.38% (RSD = 0.97%) (RSD criterion < 1.65%)
Interference/ Specificity	There are no interfering peaks (Injection of blank, prothioconazole standard solution, folpet standard solution, sample solution, blank formulation solution, impurity CCl_4, impurity PMM, impurity prothioconazole-desthio, toluene and fortified sample (with CCl_4, PMM, prothioconazole-desthio and toluene) solutions). Specific method.	
Comment	-	

Conclusion
The analytical method for the determination of active substance in the plant protection product SA2101F has been described and validated according with SANCO/3030/99 rev. 5 and accomplishes with all parameters.

5.2.1.2 Description of analytical methods for the determination of relevant impurities (KCP 5.1.1)

The approval regulation for prothioconazole (Implementing Regulation (EU) No. 540/2011) stipulates maximum limits for toluene and prothioconazole-desthio of 5 and 0.5 g/kg, respectively, in technical prothioconazole.
The approval regulation for folpet (Implementing Regulation (EU) No. 540/2011) stipulates maximum limits for perchloromethylmercaptan and carbon tetrachloride of 3.5 and 4 g/kg, respectively, in technical folpet.
An overview on the acceptable methods and possible data gaps for analysis of relevant impurities in plant protection product is provided as follows:

Comments of zRMS:	Study acceptable The analytical method for the determination of toluene, prothioconazole-desthio, PMM and CCl4 in ZELORA START was fully validated according to SANCO/3030/99 rev. 5.

Reference:	KCP 5.1.1/02
Report	PROTHIOCONAZOLE 120 g/L + FOLPET 300 g/L SC (SAP2101F): Physical, chemical and technical properties of the plant protection product, Morais, F., 2022, Report no EF/371/21 – Interim Report (T0) – Annex 3.
Guideline(s):	Yes, SANCO/3030/99 rev. 5
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Reference:	KCP 5.1.1/03
Report	PROTHIOCONAZOLE 120 g/L + FOLPET 300 g/L SC (SAP2101F): Physical, chemical and technical properties of the plant protection product, Morais, F., 2022, Report no EF/371/21 – Interim Report (T0) – Annex 4.
Guideline(s):	Yes, SANCO/3030/99 rev. 5
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Reference:	KCP 5.1.1/04
Report	PROTHIOCONAZOLE 120 g/L + FOLPET 300 g/L SC (SAP2101F): Physical, chemical and technical properties of the plant protection product, Morais, F., 2022, Report no EF/371/21 – Interim Report (T0) – Annex 5.
Guideline(s):	Yes, SANCO/3030/99 rev. 5
Deviations:	No
GLP:	Yes
Acceptability:	Yes

- **<u>Toluene and prothioconazole-desthio</u>**

Materials and methods

The sample is dissolved in acetonitrile. After that the solution is placed in an ultra-sonic bath and filtered. Toluene and prothioconazole-desthio are quantified using a HPLC-DAD method.

Chromatographic conditions – HPLC-DAD for impurities quantification

Mobile phase	Acetonitrile: 0.05% Ortho-phosphoric acid (35:65)	
Run time	60 minutes	
Flow	0.300 mL/min	
Column	Zorbax Eclipse XDB-C8; 150 mm × 2.1 mm; 3.5 µm	
Column temperature	30 ºC	
Detection wavelength	216 nm	
Injection volume	3.0 µL	
Retention time	Toluene:	Around 15.2 minutes
	Prothioconazole-desthio:	Around 17.1 minutes

Chromatographic conditions – GLC-MS/MS for impurities identification

Injector method	*Injection volume*	1.00 µL
	Pre-inj dwell time	3 s
	Post-inj dwell time	3 s
GC method	*Temperature*	40 ºC (5 min) 5 ºC/min to 300 ºC for 3 minutes
	Column	TG-5MS, 30 m × 0.25 mm × 0.25 µm film thickness
	S/SL mode	Split
	Inlet temperature	200 ºC
	Split flow	19.0 mL/min (constant flow)
	Carrier flow	1.900 mL/min
Detector Method (MS/MS)	*Temperature*	250 ºC
	Ion source	250 ºC
	Start time	7.0 minutes
	Ionization mode	EI
	Ion polarity	Positive
	Acquisition mode	SCAN (m/z 30 – 400 amu)
Retention times	*Toluene* *Prothioconazole-desthio*	Around 10.3 minutes Around 48.3 minutes

Standard solution preparation

Toluene: Weigh approximately 25 mg ± 10% of toluene reference material into a 25 mL volumetric flask. Dissolve and complete the volume with acetonitrile (1.0 mg/mL). Prepare in duplicate (solutions STD1 and STD2).

Prothioconazole-desthio: Weigh approximately 10 mg ± 10% of prothioconazole-desthio reference material into a 10 mL volumetric flask. Dissolve and complete the volume with acetonitrile (1.0 mg/mL). Prepare in duplicate (solutions STD1 and STD2).

Calibration plot preparation

From each one of the two standard stock solutions, prepare five calibration solutions accordingly to the following tables, into a final volume of acetonitrile. Combined solutions of toluene and prothioconazole-desthio can be prepared.

Toluene calibration plot preparation:

Level	Intermediate solution volume (mL)	Final volume (mL)	Final concentration (mg/mL)	Final concentration[1] (%)
L1	0.050	10	0.0050	0.0024
L2	0.120	10	0.0120	0.0056
L3	0.200	10	0.0200	0.0094
L4	0.250	10	0.0250	0.0118
L5	0.700	10	0.0700	0.0330

[1] For samples at 212 mg/mL

Prothioconazole-desthio calibration plot preparation:

Level	Intermediate solution volume (mL)	Final volume (mL)	Final concentration (mg/mL)	Final concentration[1] (%)
L1	0.050	10	0.0050	0.0024
L2	0.120	10	0.0120	0.0056
L3	0.200	10	0.0200	0.0094
L4	0.250	10	0.0250	0.0118
L5	0.700	10	0.0700	0.0330

[1] For samples at 212 mg/mL

For quantification purpose, a calibration plot can be prepared with three calibration levels in duplicate, as long as it covers ± 20 % of the nominal concentration of the analyte.

Sample solution preparation

Weigh approximately 2124 mg ± 10 mg of sample into a 10 mL volumetric flask. Dissolve and complete the volume with acetonitrile (212 mg/mL). Place the solution in an ultra-sonic bath, filter using a 0.20 µm disk filter. Prepare in duplicate.

- **Perchloromethylmercaptan (PMM)**

Materials and methods

The sample is dissolved in toluene. After that the solution is placed in an ultra-sonic bath and filtered. Perchloromethylmercaptan (PMM) is quantified using a GLC method with MS/MS detection, operating in SRM (Single Reaction Monitoring) mode.

Chromatographic conditions

Injector method	*Injection volume*	5.00 µL
	Pre-inj dwell time	3 s
	Post-inj dwell time	3 s
GC method	*Temperature*	70 ºC for 5 minutes 15.0 ºC/min until 220 ºC (maintain for 5 minutes)
	Column	TG-5MS, 30 m × 0.25 mm × 0.25 µm film thickness
	S/SL mode	Split
	Inlet temperature	280 ºC
	Split flow	7.5 mL/min (constant flow)
	Carrier flow	1.500 mL/min
Detector Method (MS/MS)	*Temperature*	280 ºC
	Ion source	230 ºC
	Start time	7.5 minutes
	Ionization mode	EI
	Ion polarity	Positive
	Acquisition mode	SRM conditions Scan #1 (SRM1) · Precursor mass: 149 amu · Q3 Start mass: 78.995 amu · Q3 End mass: 79.005 amu · Scan time: 0.2 sec · Collision energy: 20 Scan #2 (SRM2) · Precursor mass: 151 amu · Q3 Start mass: 115.995 amu · Q3 End mass: 116.005 amu · Scan time: 0.2 sec · Collision energy: 10
Retention times	*PMM*	Around 7.6 minutes

Standard solution preparation

Weigh about 50 mg ± 10% of PMM reference material into a 50 mL volumetric flask. Dissolve and complete the volume with toluene (1.0 mg/mL). From this solution transfer 0.4 mL into a 50 mL volumetric flask and complete the volume with toluene (0.008 mg/mL – intermediate solution). Prepare in duplicate (solutions STD1 and STD2).

Calibration plot preparation

From each one of the two standard stock solutions, prepare five calibration solutions accordingly to the following table, into a final volume of toluene.

Level	Intermediate solution volume (mL)	Final volume (mL)	Final concentration (mg/mL)	Final concentration[1] (%)
L1	0.15	10	0.0001	0.0488
L2	0.25	10	0.0002	0.0813
L3	0.35	10	0.0003	0.1138
L4	0.50	10	0.0004	0.1626
L5	0.70	10	0.0006	0.2276

[1] For samples at 0.25 mg/mL

For quantification purpose, a calibration plot can be prepared with three calibration levels in duplicate, as long as it covers ± 20 % of the nominal concentration of the analyte.

Sample solution preparation

Weigh approximately 123 mg ± 10 mg of test item into a 100 mL volumetric flask. Dissolve and complete the volume with toluene (1.2 mg/mL). Place the solution in an ultra-sonic bath, filter using a 0.20 µm disk filter. From this solution transfer 2.0 mL into a 10 mL volumetric flask and complete the volume with toluene (0.25 mg/mL). Prepare in duplicate.

- **Carbon tetrachloride (CCl_4)**

Materials and methods

The sample is dissolved in dichloromethane. After that the solution is placed in an ultra-sonic bath and filtered. Carbon tetrachloride (CCl_4) is quantified using a GLC method with MS/MS detection, operating in SRM (Single Reaction Monitoring) mode.

Chromatographic conditions

Injector method	*Injection volume*	5.00 µL
	Pre-inj dwell time	3 s
	Post-inj dwell time	3 s
GC method	*Temperature*	40 ºC for 4 minutes 45.0 ºC/min until 250 ºC (maintain for 17 minutes)
	Column	TG-5MS, 30 m × 0.25 mm × 0.25 µm film thickness
	S/SL mode	Split
	Inlet temperature	200 ºC
	Split flow	24.0 mL/min (constant flow)
	Carrier flow	1.200 mL/min
Detector Method (MS/MS)	*Temperature*	250 ºC
	Ion source	250 ºC
	Start time	3.2 min (filament on) 5.0 min (filament off)
	Ionization mode	EI
	Ion polarity	Positive
	Acquisition mode	SRM conditions Scan #1 (SRM1) · Precursor mass: 82 amu · Q3 Start mass: 46.995 amu · Q3 End mass: 47.005 amu

		· Scan time: 0.2 sec · Collision energy: 20 Scan #2 (SRM2) · Precursor mass: 117 amu · Q3 Start mass: 81.995 amu · Q3 End mass: 82.005 amu · Scan time: 0.2 sec · Collision energy: 10
Retention times	*CCl_4*	Around 3.6 minutes

Standard solution preparation

Weigh about 100 mg ± 10 mg of CCl_4 reference material into a 100 mL volumetric flask. Dissolve and complete the volume with dichloromethane (1.0 mg/mL). From this solution transfer 0.4 mL into a 50 mL volumetric flask and complete the volume with dichloromethane (0.008 mg/mL – intermediate solution). Prepare in duplicate (solutions STD1 and STD2).

Calibration plot preparation

From each one of the two standard stock solutions, prepare five calibration solutions accordingly to the following table, into a final volume of dichloromethane.

Level	Intermediate solution volume (mL)	Final volume (mL)	Final concentration (mg/mL)	Final concentration[1] (%)
L1	0.10	10	0.00008	0.033
L2	0.15	10	0.00012	0.049
L3	0.25	10	0.00020	0.081
L4	0.40	10	0.00032	0.130
L5	0.70	10	0.00056	0.228

[1] For samples at 0.25 mg/mL

For quantification purpose, a calibration plot can be prepared with three calibration levels in duplicate, as long as it covers ± 20 % of the nominal concentration of the analyte.

Sample solution preparation

Weigh approximately 123 mg ± 10 mg of test item into a 100 mL volumetric flask. Dissolve and complete the volume with dichloromethane (1.2 mg/mL). Place the solution in an ultra-sonic bath, filter using a 0.20 µm disk filter. From this solution transfer 2.0 mL into a 10 mL volumetric flask and complete the volume with dichloromethane (0.25 mg/mL). Prepare in duplicate.

Validation - Results and discussions

Tables 5.2-2: Methods suitable for the determination of the relevant impurities in plant protection product (PPP) SAP2101F

	Toluene max. 0.6 g/L	**Prothioconazole-desthio max. 0.06 g/L**
Author(s), year	Morais, F., 2022	Morais, F., 2022
Principle of method	HPLC-DAD	HPLC-DAD
Linearity (linear between mg/L) (correlation coefficient, expressed as r)	Range: [0.0023% - 0.0330%], [0.0050 – .0700 mg/mL] y=32836.4591x – 16.2773 r = 0.9999	Range: [0.0023% - 0.0324%], [0.0049 – 0.0689 mg/mL] y=20209.6255x – 43.1828 r = 0.9997
Precision – Repeatability Mean n = 5 (%RSD)	System rep.: RSD = 1.14%, Hr = 0.20 Method rep.: RSD = 0.32%, Hr = 0.05 (expected content: 0.006% w/w, RSD criterion < 5.79%)	System rep.: RSD = 1.88%, Hr = 0.32 Method rep.: RSD = 1.10%, Hr = 0.19 (expected content: 0.006% w/w, RSD criterion < 5.79%)

	Toluene **max. 0.6 g/L**	**Prothioconazole-desthio** **max. 0.06 g/L**
Accuracy **n = 5** **(% Recovery)** **Total Recovery**	1st level(0.006%): 102.36% (RSD=0.45%) (RSD criterion < 5.79%) 2nd level (0.012%): 89.33% (RSD=0.54%) (RSD criterion < 5.21%)	1st level(0.006%): 107.72% (RSD=1.45%) (RSD criterion < 5.79%) 2nd level(0.012%): 102.14% (RSD=1.57%) (RSD criterion < 5.21%)
Interference/ Specificity	There are no interfering peaks (Injection of blank, toluene standard solution, prothioconazole-desthio standard solution, sample solution, blank formulation solution, impurity PMM, impurity CCl_4 and fortified sample solutions). Specific method.	
LOQ	LOQ=0.006% w/w	LOQ=0.006% w/w
Comment	Result: < LOQ (0.006%)	Result: < LOQ (0.006%)

	PMM **max. 1.05 g/L**	**CCl_4** **max. 2 g/L**
Author(s), year	Morais, F., 2022	Morais, F., 2022
Principle of method	GLC MS/MS	GLC MS/MS
Linearity **(linear between mg/L)** **(correlation coefficient, expressed as r)**	Range: [0.064% - 0.226%], [0.000158 – 0.000555 mg/mL] y=7645035889.4x – 323942.8 r = 0.9961	Range: [0.0323% - 0.2276%], [0.000080 – 0.000560 mg/mL] y = 34460581304.5x – 893005.9 r = 0.9980
Precision – Repeatability **Mean** **n = 5** **(%RSD)**	System rep.: RSD = 2.69%, Hr = 0.69 Method rep.: RSD = 2.35%, Hr = 0.60 (expected content: 0.08% w/w, RSD criterion < 3.92%)	System rep.: RSD = 1.22%, Hr = 0.29 Method rep.: RSD = 0.89%, Hr = 0.21 (expected content: 0.05% w/w, RSD criterion < 4.21%)
Accuracy **n = 5** **(% Recovery)** **Total Recovery**	1st level (0.08%): 98.47% (RSD = 1.84%) (RSD criterion < 3.92%) 2nd level (0.16%): 100.10% (RSD=0.71%) (RSD criterion < 3.53%)	1st level (0.05%): 106.95% (RSD = 0.71%) (RSD criterion < 4.21%) 2nd level (0.13%): 101.46% (RSD=1.46%) (RSD criterion < 3.64%)
Interference/ Specificity	There are no interfering peaks (Injection of blank, PMM standard solution, sample solution, blank formulation solution, impurity prothioconazole-desthio, toluene, impurity CCl_4 and fortified sample solutions). Specific method.	There are no interfering peaks (Injection of blank, CCl_4 standard solution, sample solution, blank formulation solution, impurity prothioconazole-desthio, toluene, impurity PMM and fortified sample solutions). Specific method.
LOQ	LOQ=0.080% w/w	LOQ=0.050% w/w
Comment	Result: < LOQ (0.080%)	Result: < LOQ (0.050%)

Conclusion
The analytical methods for the determination of toluene, prothioconazole-desthio, PMM and CCl_4 in the plant protection product SA2101F has been described and validated according with SANCO/3030/99 rev. 5 and accomplishes with all parameters.

5.2.1.3 Description of analytical methods for the determination of formulants (KCP 5.1.1)

There are no formulants or constituents of formulants within the preparation or formed during storage, that are of toxicological, ecotoxicological or environmental relevance. Therefore, this point is not relevant.

5.2.1.4 Applicability of existing CIPAC methods (KCP 5.1.1)

There are no CIPAC methods available for the quantification of prothioconazole and folpet in suspension concentrate applicable to SAP2101F.

5.2.2 Methods for the determination of residues (KCP 5.1.2)

An overview on the acceptable methods and possible data gaps for analysis of residues of prothioconazole and folpet for the generation of pre-authorization data is given in the following tables. For the detailed evaluation of new/additional studies it is referred to Appendix 2.

Table 5.2-3: Validated methods for the generation of pre-authorization data

Component of residue definition: prothioconazole, prothioconazole-desthio (M04) and TA, TLA, TAA and 1,2,4-triazole				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
Wheat and barley (Residues) Prothioconazole-desthio	Primary	0.01 mg/kg	HPLC-MS/MS	Heinemann, 2001a (Bayer method 00647)
	Confirmatory (if required)	-	-	Not required
~~Oilseed rape (dry matrices: straw) (Residues)~~ ~~TA, TLA, TAA and 1,2,4-triazole~~	~~Primary~~	~~0.01 mg/kg for all analytes~~	~~LC-DMS/MS/MS~~	~~KCP 5.1.2/01 Stolze, J. 2022~~
	~~Confirmatory (if required)~~	~~-~~	~~-~~	~~Not required~~
Muscle, liver, kidney and fat (Residues) Prothioconazole-desthio	Primary	0.01 mg/kg	HPLC-MS/MS	Heinemann, O. (2001) / EU agreed Report no: 00655
	Confirmatory (if required)	-	-	Not required
Milk (Residues) Prothioconazole desthio	Primary	0.004 mg/kg	HPLC-MS/MS	Heinemann, O. (2001) / EU agreed Report no: 00655 Heinemann, O. (2001) / EU agreed Report no: 00655/M001
	Confirmatory (if required)	-	-	Not required
Milk, muscle, liver, fat, kidney and egg (Residues) TA, TLA, TAA and 1,2,4-triazole	Primary	0.01 mg/kg	LC-MS/MS	Billian, P. Druskus, M (2009)
	Confirmatory (if required)	-	-	Not required
Soil, water, sediment,... (Environmental fate)	Primary	-	-	No new methods submitted
	Confirmatory (if required)	-	-	No new methods submitted
Soil, water,... (Efficacy)	Primary	-	-	No new methods submitted
	Confirmatory (if required)	-	-	No new methods submitted
Feed, body fluids,... (Toxicology)	Primary	-	-	No new methods submitted
	Confirmatory (if required)	-	-	No new methods submitted
Body fluids, air,.... (Exposure)	Primary	-	-	No new methods submitted
	Confirmatory (if required)	-	-	No new methods submitted
Test water (Ecotoxicology)	Primary	0.00849 mg/L	HPLC-MS/MS	KCP 5.1.2/02 Schuler, 2022 / New study

Component of residue definition: prothioconazole, prothioconazole-desthio (M04) and TA, TLA, TAA and 1,2,4-triazole				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
Prothioconazole				
Test water (Ecotoxicology) Prothioconazole	Primary	0.00948 mg/L	HPLC-MS/MS	KCP 5.1.2/03 Schuler, 2022 / New study)
Application solution (Ecotoxicology) Prothioconazole	Primary	149 mg/L	LC-MS/MS	KCP 5.1.2/04 Lingott, 2022 /New study
Application solution (Ecotoxicology) Prothioconazole	Primary	149 mg/L	LC-MS/MS	KCP 5.1.2/05 Lingott, 2022 /New study
Application solution (Ecotoxicology) Prothioconazole	Primary	0.994 mg/Kg	LC-MS/MS	KCP 5.1.2/06 Rastogi T. 2022 /New study
Water, buffer solutions,... (Properties)	Primary	-	-	No new methods submitted
	Confirmatory (if required)	-	-	No new methods submitted

Component of residue definition: sum of folpet and phthalimide expressed as folpet, phtalamide expressed as folpet and folpet.				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
Wheat and Barley (Residues) Sum of folpet and phtalimide, expressed as folpet.	Primary	Folpet: 0.01mg/kg Phtalimide: Grain&Whole Plant: 0.01 mg/kg Straw: 0.05 mg/kg	LC MS/MS LC-QTRAP	Jooß, S., 2022/ New study (KCP 5.1.2/07) Gordo, J, 2022/ New study (KCP 5.1.2/10)
Processed comodities		Folpet (each matrix): 0.01mg/kg Phtalimide (each matrix): 0.01 mg/kg Phthalic Acid (each matrix): 0.05 mg/kg Phthalamic Acid (each matrix): 0.05 mg/kg	LC-MS/MS	Jooß, S., 2022/ New study (KCP 5.1.2/08)
	Confirmatory (if required)	-	-	Not required
Animal products, food of animal origin,... (Residues) Phthalimide expressed as folpet	Primary	-	-	Not required (refer to Part B Section 7)
	Confirmatory (if required)	-	-	Not required (refer to Part B Section 7)

Component of residue definition: sum of folpet and phthalimide expressed as folpet, phtalamide expressed as folpet and folpet.				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
Soil, water, sediment,... (Environmental fate)	Primary	-	-	No new methods submitted
	Confirmatory (if required)	-	-	No new methods submitted
Soil, water,... (Efficacy)	Primary	-	-	No new methods submitted
	Confirmatory (if required)	-	-	No new methods submitted
Feed, body fluids,... (Toxicology)	Primary	-	-	No new methods submitted
	Confirmatory (if required)	-	-	No new methods submitted
Body fluids, air,.... (Exposure)	Primary	-	-	No new methods submitted
	Confirmatory (if required)	-	-	No new methods submitted
Test water. (Ecotoxicology) Folpet	Primary	0.0226 mg/L	HPLC-MS/MS	KCP 5.1.2/02 Schuler, 2022 / New study
Test water. (Ecotoxicology) Folpet	Primary	0.0253 mg/L	HPLC-MS/MS	KCP 5.1.2/03 Schuler, 2022 / New study
Application solution (Ecotoxicology) Folpet	Primary	398 mg/L	LC-MS/MS	KCP 5.1.2/04 Lingott J., 2022 /New study
Application solution (Ecotoxicology) Folpet	Primary	398 mg/L	LC-MS/MS	KCP 5.1.2/05 Lingott J., 2022 /New study
Feeding solution (Ecotoxicology) Folpet	Primary	2.65 mg/kg	LC-MS/MS	KCP 5.1.2/06 Rastogi T. 2022 /New study
Feeding solution (Ecotoxicology) Folpet	Primary	30.10 mg	HPLC - UV	KCP 5.1.2/09 Schreitmüller J. 2016 / New study
Water, buffer solutions,... (Properties)	Primary	-	-	No new methods submitted
	Confirmatory (if required)	-	-	No new methods submitted

zRMS comments:
New analytical methods for the determination of prothioconazole and folpet for the generation of pre-authorization data have been submitted by Applicant. The detailed of the methods are presented in Appendix 2.

5.3 Methods for post-authorization control and monitoring purposes (KCP 5.2)

5.3.1 Analysis of the plant protection product (KCP 5.2)

Analytical methods for the determination of the active substance and relevant impurities in the plant protection product shall be submitted, unless the applicant shows that these methods already submitted in accordance with the requirements set out in point 5.2.1 can be applied.

The methods already submitted in accordance with the requirements set out in point 5.2.1 can be applied for post-authorization and monitoring and therefore additional methods under this point have not been submitted.

5.3.2 Description of analytical methods for the determination of residues of prothioconazole (KCP 5.2)

5.3.2.1 Overview of residue definitions and levels for which compliance is required

Compared to the residue definition proposed in the Draft Assessment Report (incl. its addenda) the current legal residue definition is not identical.

Table 5.3-1: Relevant residue definitions for monitoring/enforcement and levels for which compliance is required

Matrix	Residue definition	MRL / limit	Reference for MRL/level Remarks
Plant, high water content	Prothioconazole-desthio	0.01* mg/kg	Reg. (EU) ~~2019/552~~ 2024/1318
Plant, high acid content		0.01* mg/kg	Reg. (EU) ~~2019/552~~ 2024/1318
Plant, high protein/high starch content (dry commodities)		0.01* mg/kg	Reg. (EU) ~~2019/552~~ 2024/1318
Plant, high oil content		0.01* mg/kg	Reg. (EU) ~~2019/552~~ 2024/1318
Plant, difficult matrices (hops, spices, tea)		0.05* mg/kg	Reg. (EU) ~~2019/552~~ 2024/1318
Muscle	Prothioconazole-desthio	0.01* mg/kg	Reg. (EU) ~~2019/552~~ 2024/1318
Milk		0.01* mg/kg	Reg. (EU) ~~2019/552~~ 2024/1318
Eggs		0.01* mg/kg	Reg. (EU) ~~2019/552~~ 2024/1318
Fat		0.01* mg/kg	Reg. (EU) ~~2019/552~~ 2024/1318
Liver, kidney		0.5 mg/kg	Reg. (EU) ~~2019/552~~ 2024/1318
Soil (Ecotoxicology)	Prothioconazole and prothioconazole-desthio	0.05 mg/kg	common limit
Drinking water (Human toxicology)	Prothioconazole and prothioconazole-desthio	0.1 µg/L	general limit for drinking water
Surface water (Ecotoxicology)	Prothioconazole	308 µg/L	Lowest NOEC from aquatic toxicity studies (*Onchorhynchus mykiss (ELS)*)
	Prothioconazole-desthio	3.34 µg/L	Lowest NOEC from aquatic toxicity studies (*Onchorhynchus mykiss (ELS)*)
Air	Prothioconazole	60 µg/m^3	AOEL sys/AOEL inhal: 0.2 mg/kg bw/d
	Prothioconazole-desthio	3 µg/m^3	AOEL sys/AOEL inhal: 0.01 mg/kg bw/d

Matrix	Residue definition	MRL / limit	Reference for MRL/level Remarks
Tissue (meat or liver)	Not applicable (EFS, 2007) prothioconazole-desthio	not required (EFSA, 2007) 0.01 mg/kg	not classified as T / T+ (EFSA, 2007) General limit according to SANTE/2020/12830, Rev.2
Body fluids		not required (EFSA, 2007) 0.01 mg/L	not classified as T / T+ (EFSA, 2007) General limit according to SANTE/2020/12830, Rev.2

(*) MRLs proposed at the LOQ.

zRMS comments:
The Reg. (EU) 2024/1318 for prothioconazole is now in force.

Triazole Derived Metabolites:
MRLs are not set for the triazole derivative metabolites and as such monitoring methods are not required.

5.3.2.2 Description of analytical methods for the determination of residues in plant matrices (KCP 5.2)

An overview on the acceptable methods and possible data gaps for analysis of prothioconazole in plant matrices is given in the following tables. For the detailed evaluation of new/additional studies it is referred to Appendix 2.

Table 5.3-2: Validated methods for food and feed of plant origin (required for all matrix types, "difficult" matrix only when indicated by intended GAP)

Component of residue definition: Prothioconazole-desthio (M04)				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
High water content	Primary	0.02 mg/kg	GC/MS	Weeren, R. D., Pelz, S. (2000) / EU agreed Report no: 00086/M033
	ILV	0.02 mg/kg	GC/MS	Class, Th. (2001) / EU agreed Report no: P/B 484 G
	Confirmatory (if required)	-	-	-
High acid content	Primary	0.02 mg/kg	GC/MS	Weeren, R. D., Pelz, S. (2000) / EU agreed Report no: 00086/M033
	ILV	0.02 mg/kg	GC/MS	Class, Th. (2001) / EU agreed Report no: P/B 484 G
	Confirmatory (if required)	-	-	-
High oil content	Primary	0.02 mg/kg	GC/MS	Weeren, R. D., Pelz, S. (2000) / EU agreed Report no: 00086/M033
	ILV	-	-	-
	Confirmatory (if required)	-	-	-
High protein/high starch content	Primary	0.02 mg/kg	GC/MS	Weeren, R. D., Pelz, S. (2000) / EU agreed

Component of residue definition: Prothioconazole-desthio (M04)				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
(dry)				Report no: 00086/M033
	ILV	0.02 mg/kg	GC/MS	Class, Th. (2001) / EU agreed Report no: P/B 484 G
	Confirmatory (if required)	0.01 mg/kg	HPLC-MS/MS	Heinemann, O. (2000) / EU agreed Report no: 00598
Difficult (if required, depends on intended use)	Primary	0.02 mg/kg	GC/MS	Weeren, R. D., Pelz, S. (2000) / EU agreed Report no: 00086/M033
	ILV	-	-	-
	Confirmatory (if required)	-	-	-

For any special comments or remarkable points concerning the analytical methods for the determination of residues in plant matrices, please refer to Appendix 2.

Table 5.3-3: Statement on extraction efficiency

	Method for products of plant origin
Required, available from:	Required. Available from Weeren, Pelz 2000 (00086/M033, DFG Method S19) "The extraction method used in the metabolism study is comparable to the one validated in the Weeren/Pelz (2000) study, that has demonstrated suitability for the analysis of cereal grain and straw. While the first one uses acetonitrile/water for extraction, the second one uses acetone/water. Acetonitrile and acetone are both polar protic solvents, with very similar polarity value (5.1 for acetone and 5.8 for acetonitrile). In conclusion, both extractions are comparable and extraction efficiency is demonstrated. No additional data is required."

zRMS comments:
According to the EFSA Journal 2014;12(5):3689:
Methods for enforcement of residues in food of plant origin
During the peer review under Directive 91/414/EEC, an analytical method using GC-MS and its ILV were evaluated and validated for the determination of prothioconazole-desthio in plant matrices with an LOQ of 0.02 mg/kg in high water content (tomato), high oil content (rape seed), acidic (orange), dry (wheat grain) commodities and an LOQ of 0.05 mg/kg in straw. This method can be confirmed by an independent analytical method using HPLC-MS/MS fully validated for the determination of prothioconazole-desthio in high water content commodities and in straw with an LOQ of 0.05 mg/kg and in high oil content and in dry commodities with an LOQ of 0.01 mg/kg (United Kingdom, 2004). The analytical methods are not enantioselective, hence the sum of isomers will be analyzed.

The multi-residue QuEChERS method in combination with HPLC-MS/MS, as described by CEN (2008), is also available to analyse the prothioconazole-desthio in plant commodities. Nevertheless, the validation data reported are too limited to conclude on the validity of this analytical method (EURL, 2013).
Hence it is concluded that prothioconazole-desthio can be enforced in food of plant origin with an LOQ of 0.02 mg/kg in high oil content and dry commodities and an LOQ of 0.05 mg/kg in high water content commodities and in straw taking into account the highest LOQ of both methods.

Since many MRLs have been lowered to 0.01 mg/kg, the validated LOQ of the EU agreed methods by Weeren and Pelz (2000) and Class (2001) is not sufficient to monitor these lowered MRLs for food of plant origin. Analytical methods with appropriate ILVs with a lower LOQ value for plant matrices should be provided.
At the request of the evaluator, the applicant provided a response:
Applicants reply: *The applicant agreed that new studies with appropriate LOQ should be provided. The active substance is under renewal and the applicant is preparing a DMT to be submitted. However, the monitoring methods have not started yet since we are waiting on the EFSA publication confirming or amending the residue*

definition available in RAR. Since this is the next step in the renewal process applicant requests to submit the method as confirmatory data as soon as possible.

zRMS-PL: In our opinion analytical methods with appropriate ILVs for the determination of prothioconazole in all major matrix groups with an LOQ of 0.01 mg/kg is required according to the requirement of SANTE/2020/12830, Rev.2, 14. February 2023 and should be provided as a post-registration requirement.

5.3.2.3 Description of analytical methods for the determination of residues in animal matrices (KCP 5.2)

An overview on the acceptable methods and possible data gaps for analysis of prothioconazole in animal matrices is given in the following tables. For the detailed evaluation of new/additional studies it is referred to Appendix 2.

Table 5.3-4: Validated methods for food and feed of animal origin (if appropriate)

Component of residue definition: Prothioconazole-desthio (M04)				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (*i.e.* GC-MS or HPLC-UV)**	**Author(s), year / missing**
Milk	Primary	0.01 mg/kg 0.004 mg/kg	HPLC-MS/MS HPLC-MS/MS	Heinemann, O. (2001) / EU agreed Report no: 00655 Heinemann, O. (2001) / EU agreed Report no: 00655/M001
	ILV	0.004 mg/kg	HPLC-MS/MS	Dubey, L. (2001) / EU agreed Report no: A-14-01-01
	Confirmatory (if required)	-	-	-
Eggs	Primary	Not required.	-	-
	ILV	-	-	-
	Confirmatory (if required)	-	-	-
Muscle	Primary	0.02 mg/kg	HPLC-MS/MS	Heinemann, O. (2001) / EU agreed Report no: 00655
	ILV	0.004 mg/kg	HPLC-MS/MS	Dubey, L. (2001) / EU agreed Report no: A-14-01-01
	Confirmatory (if required)	-	-	-
Fat	Primary	0.03 mg/kg	HPLC-MS/MS	Heinemann, O. (2001) / EU agreed Report no: 00655
	ILV	0.004 mg/kg	HPLC-MS/MS	Dubey, L. (2001) / EU agreed Report no: A-14-01-01
	Confirmatory (if required)	-	-	-
Kidney, liver (offal)	Primary	0.04 mg/kg	HPLC-MS/MS	Heinemann, O. (2001) / EU agreed Report no: 00655
	ILV	0.004 mg/kg	HPLC-MS/MS	Dubey, L. (2001) / EU agreed Report no: A-14-01-01
	Confirmatory (if required)	-	-	-

For any special comments or remarkable points concerning the analytical methods for the determination of

residues in animal matrices, please refer to Appendix 2.

Table 5.3-5: Statement on extraction efficiency

	Method for products of animal origin
Required, available from:	Required. The extraction efficiency of the residue method in animal matrices was previously demonstrated for the Annex I inclusion by Heinemann, O.; "ANALYTICAL DETERMINATION OF RESIDUES OF JAU6476-3-HYDROXYDESTHIO, JAU6476-4-HYDROXY-DESTHIO, AND JAU6476-DESTHIO IN/ON MATRICES OF ANIMAL ORIGIN BY HPLC-MS/MS"; document M-037709-01-1, (please refer to KIIA 4.2.1.1 from original Annex I inclusion) using aged radioactive residues from the goat metabolism study (Weber, H., Weber, E. and Spiegel, K.; document M-042103-01-1, please refer to KIIA 6.2.2.2. from original Annex I inclusion). In summary, the comparison of the residue analytical method of extraction for animal matrices with the extraction method used in the metabolism study demonstrated the suitability of the analytical method (extracting with an acetonitrile/water solvent system) for the determination of the relevant residue in animal matrices. No further consideration is necessary.

zRMS comments:
According to the EFSA Journal 2014;12(5):3689:
Methods for enforcement of residues in food of animal origin
During the peer review under Directive 91/414/EEC, an analytical method using HPLC-MS/MS and its ILV were evaluated and validated for the determination of prothioconazole-desthio only in food of animal origin with an LOQ of 0.004 mg/kg in milk and an LOQ of 0.01 mg/kg in muscle, fat, liver and kidney (United Kingdom, 2004; EFSA, 2007b). Hence it is concluded that prothioconazole-desthio can be enforced in food of animal origin with an LOQ of 0.004 mg/kg in milk and an LOQ of 0.01 mg/kg in muscle, fat, liver and kidney. Nevertheless, prothioconazole-desthio cannot be enforced in eggs. Therefore, ***a fully validated analytical method for the determination of prothioconazole-desthio in eggs is required.***
The available analytical method is not enantioselective, hence the sum of isomers will be analyzed.

Since many MRLs have been lowered to 0.01 mg/kg, the validated LOQ of the EU agreed methods by Heinemann, O. (2001) is not sufficient to monitor these lowered MRLs for animal origin. Analytical methods with appropriate ILVs with a lower LOQ value for animal matrices should be provided.
Additionally according to EFSA's findings presented in the EFSA Journal 2014; 12(5):3689, validation of the method for the determination of prothioconazole-desthio in eggs is also required.

At the request of the evaluator, the applicant provided a response:
Applicants reply: *The applicant agreed that new studies with appropriate LOQ should be provided. The active substance is under renewal and the applicant is preparing a DMT to be submitted. However, the monitoring methods have not started yet since we are waiting on the EFSA publication confirming or amending the residue definition available in RAR. Since this is the next step in the renewal process applicant requests to submit the method as confirmatory data as soon as possible.*

zRMS-PL: In our opinion analytical methods with ILVs with appropriate LOQ for the determination of prothioconazole in animal matrices is required according to the requirement of SANTE/2020/12830, Rev.2, 14. February 2023 and should be provided as a post-registration requirement.

5.3.2.4 Description of methods for the analysis of soil (KCP 5.2)

An overview on the acceptable methods and possible data gaps for analysis of prothioconazole in soil is given in the following tables. For the detailed evaluation of new/additional studies it is referred to Appendix 2.

Table 5.3-6: Validated methods for soil (if appropriate)

Component of residue definition: prothioconazole, prothioconazole-desthio (M04)			
Method type	**Method LOQ**	**Principle of method (*i.e.* GC-MS or HPLC-UV)**	**Author(s), year / missing**
Primary	0.006 mg/kg	HPLC-MS/MS	Schramel, O. (2000) / EU agreed Report no: 00610
Confirmatory	0.01 mg/kg	GC/MS	Steinhauer, S. (2001) / EU agreed Report no: 00086/M038

For any special comments or remarkable points concerning the analytical methods for soil please refer to Appendix 2.

zRMS comments:
Analytical method is available to determine residues of prothioconazole-desthio and prothioconazole in soil (EFSA, 2007).

5.3.2.5 Description of methods for the analysis of water (KCP 5.2)

An overview on the acceptable methods and possible data gaps for analysis of prothioconazole in surface and drinking water is given in the following tables. For the detailed valuation of new/additional studies it is referred to Appendix 2.

Table 5.3-7: Validated methods for water (if appropriate)

Component of residue definition: prothioconazole and prothioconazole-desthio (M04)				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
Drinking water	Primary	6 µg/L	HPLC-UV	Sommer, H. (1999) / EU agreed Report no: 00586
	ILV	To be submitted at active substance level.		
	Confirmatory	0.1 µg/L (prothioconazole) 0.05 µg/L (M04)	HPLC-MS/MS	Sommer, H. (2001b) / EU agreed Report no: 00684
Surface water	Primary	6 µg/L	HPLC-UV	Sommer, H. (1999) / EU agreed Report no: 00586
	Confirmatory	0.1 µg/L (prothioconazole) 0.05 µg/L (M04)	HPLC-MS/MS	Sommer, H. (2001b) / EU agreed Report no: 00684

For any special comments or remarkable points concerning the analytical methods for water please refer to Appendix 2.

zRMS comments:
Analytical method is available to determine residues of prothioconazole-desthio and prothioconazole in water (EFSA, 2007).
An independent laboratory validation (ILV) for the method for the determination of residues of prothioconazole in drinking water is missing. Based on the indication of the SANTE/2020/12830, Rev.2 14. February 2023, the ILV for drinking water should be submitted (data gap).

Applicants reply: *The applicant agreed with RMS. The active substance is under renewal and the applicant is preparing a DMT to be submitted. However, the monitoring methods have not started yet since we are waiting for the EFSA publication confirming or amending the residue definition available in RAR. Since this is the next step in the renewal process applicant requests to submit the method as confirmatory data as soon as possible.*

zRMS-PL: It is necessary to supply the above-mentioned method for determining the residues of prothioconazole in water at the renewal of the active substance and/or re-evaluation of plant production product.

5.3.2.6 Description of methods for the analysis of air (KCP 5.2)

An overview on the acceptable methods and possible data gaps for analysis of prothioconazole in air is given in the following tables. For the detailed evaluation of new/additional studies please refer to Appendix 2.

Table 5.3-8: Validated methods for air (if appropriate)

Component of residue definition: prothioconazole, prothioconazole-desthio (M04)			
Method type	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
Primary	0.015 µg/m^3	HPLC-MS/MS	Maasfeld, W., 2002 / EU agreed Report no: 00724
	0.0006 µg/m^3	HPLC-MS/MS	Maasfeld, W., 2002 / EU agreed Report no: 00731
Confirmatory	According to SANTE/2020/12830 rev. 2 *if the analytical dectection technique of the method matches that used in either soil or water, analytical methods and either of these methods demonstrate suitable confirmatory methods, no further confirmatory information is required for air methods.*		

For any special comments or remarkable points concerning the analytical methods for air it is referred to Appendix 2.

zRMS comments:
Analytical method is available to determine residues of prothioconazole-desthio and prothioconazole in air (EFSA, 2007).

5.3.2.7 Description of methods for the analysis of body fluids and tissues (KCP 5.2)

An overview on the acceptable methods and possible data gaps for analysis of prothioconazole in body fluids and tissues is given in the following table. For the detailed evaluation of new/additional studies it is referred to Appendix 2.

Table 5.3-9: Methods for body fluids and tissues (if appropriate)

Component of residue definition: not applicable.			
Method type	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
Primary	Not required.	-	-
Confirmatory	Not required.	-	-

For any special comments or remarkable points concerning the analytical methods for body fluids and tissues please refer to Appendix 2.

zRMS comments:
According to the EFSA Scientific Report (2007) 106, 1-98, Conclusion on the peer review of Prothioconazole, the point regarding analytical methods for body fluids and tissues for prothioconazole is open, data will be required if ECB classify the active substance as toxic.
The active substance prothioconazole was evaluated at the EU level according to the old data requirements. The Commission Regulation (EU) No 284/2013 is applicable now.
In Regulation (EU) No 283/2013 it is stated that "…*methods, with a full description, shall be submitted for the*

analysis in body fluids and tissues for the active substance and relevant metabolites" and this is a new requirement of SANTE/2020/12830. According to the SANTE/2020/12830: "*Analytical methods for monitoring residues in body fluids and tissues are required for detection of active substances and/or metabolites in humans and animals after possible intoxications or for biomonitoring purposes, regardless of their toxicological classification*." Therefore, an analytical method for the residues of prothioconazole in body fluids and tissues is required (data gap).

Applicants reply: *The applicant agreed with RMS. The active substance is under renewal and the applicant is preparing a DMT to be submitted. However, the monitoring methods have not started yet since we are waiting for the EFSA publication confirming or amending the residue definition available in RAR. Since this is the next step in the renewal process applicant requests to submit the method as confirmatory data as soon as possible.*

zRMS-PL: It is necessary to supply the above-mentioned method for determining the residues of prothioconazole in body fluids at the renewal of the active substance and/or re-evaluation of plant production product (data gap).

5.3.3 Description of analytical methods for the determination of residues of folpet (KCP 5.2)

5.3.3.1 Overview of residue definitions and levels for which compliance is required

Compared to the residue definition proposed in the Draft Assessment Report (incl. its addenda) the current legal residue definition is not identical.

Table 5.3-10: Relevant residue definitions for monitoring/enforcement and levels for which compliance is required

Matrix	Residue definition	MRL / limit	Reference for MRL/level Remarks
Plant, high water content	Sum of folpet and phtalimide, expressed as folpet	0.03* mg/kg	~~Reg. (EU) 2022/93~~ Reg. (EU) 2023/1042
Plant, high acid content		0.03* mg/kg	~~Reg. (EU) 2022/93~~ Reg. (EU) 2023/1042
Plant, high protein/high starch content (dry commodities)		0.07* mg/kg	~~Reg. (EU) 2022/93~~ Reg. (EU) 2023/1042
Plant, high oil content		0.07* mg/kg	~~Reg. (EU) 2022/93~~ Reg. (EU) 2023/1042
Plant, difficult matrices (hops, spices, tea)		0.1* mg/kg	~~Reg. (EU) 2022/93~~ Reg. (EU) 2023/1042
Muscle	Phthalimide, expressed as folpet	0.05* mg/kg	~~Reg. (EU) 2022/93~~ Reg. (EU) 2023/1042
Milk		0.05* mg/kg	~~Reg. (EU) 2022/93~~ Reg. (EU) 2023/1042
Eggs		0.05* mg/kg	~~Reg. (EU) 2022/93~~ Reg. (EU) 2023/1042
Fat		0.05* mg/kg	~~Reg. (EU) 2022/93~~ Reg. (EU) 2023/1042
Liver, kidney		0.05* mg/kg	~~Reg. (EU) 2022/93~~ Reg. (EU) 2023/1042
Soil (Ecotoxicology)	Folpet	0.05 mg/kg	common limit
Drinking water (Human toxicology)	Folpet	0.1 µg/L	general limit for drinking water
Surface water (Ecotoxicology)	Folpet	39 µg/L	Lowest NOEC from fish study (*Addendum to Folpet DAR, 2005*)
Air	Folpet	30 µg/m^3	AOEL sys/AOEL inhal: 0.1 mg/kg bw/d
Tissue (meat or liver)	Not applicable (EFSA, 2009) No residue definition for body fluids/tissue is set (RAR,	Not required (EFSA, 2009) 0.01 mg/kg	Not classified as T / T+ (EFSA, 2009) General limit according to SANTE/2020/12830, Rev.2

Matrix	Residue definition	MRL / limit	Reference for MRL/level Remarks
Body fluids	2018)	Not required (EFSA, 2009) 0.01 mg/L	Not classified as T / T+ (EFSA, 2009) General limit according to SANTE/2020/12830, Rev.2

(*) MRLs proposed at the LOQ.

zRMS comments:
The Reg. (EU) 2023/1042 for folpet is now in force. Additional information has been added in Table 5.3-1.

5.3.3.2 Description of analytical methods for the determination of residues in plant matrices (KCP 5.2)

An overview on the acceptable methods and possible data gaps for analysis of folpet in plant matrices is given in the following tables. For the detailed evaluation of new/additional studies it is referred to Appendix 2.

Table 5.3-11: Validated methods for food and feed of plant origin (required for all matrix types, "difficult" matrix only when indicated by intended GAP)

Component of residue definition: Sum of folpet and phtalamide, expressed as folpet				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
High water content	Primary	0.01 mg/kg	LC-MS/MS	Perny (2015) / new study under EU review Report no R B4225
	ILV	0.01 mg/kg	LC-MS/MS	Meseguer (2016) / new study under EU review Report no S14-05779
	Confirmatory (if required)	0.01 mg/kg	LC-MS/MS	Perny (2015) / new study under EU review Report no R B4225
High acid content	Primary	0.01 mg/kg	LC-MS/MS	Perny (2015) / new study under EU review Report no R B4225
	ILV	0.01 mg/kg	LC-MS/MS	Meseguer (2016) / new study under EU review Report no S14-05779
	Confirmatory (if required)	0.01 mg/kg	LC-MS/MS	Perny (2015) / new study under EU review Report no R B4225
High oil content	Primary	0.01 mg/kg	LC-MS/MS	Wiesner, Breyer (2016) / new study under EU review Report no S16-00559 (BEL-1601V)
	ILV	0.01 mg/kg	LC-MS/MS	Hegmanns (2016) / new study under EU review Report no S16-00716
	Confirmatory (if required)	0.01 mg/kg	LC-MS/MS	Wiesner, Breyer (2016) / new study under EU review Report no S16-00559 (BEL-1601V)
High protein/high starch content	Primary	0.01 mg/kg	LC-MS/MS	Wisner, Breyer (2016) / new study under EU review

Component of residue definition: Sum of folpet and phtalamide, expressed as folpet				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
(dry)				Report no S16-00559 (BEL-1601V)
	ILV	0.01 mg/kg	LC-MS/MS	Hegmanns (2016) / new study under EU review Report no S16-00716
	Confirmatory (if required)	0.01 mg/kg	LC-MS/MS	Wiesner, Breyer (2016) / new study under EU review Report no S16-00559 (BEL-1601V)
Difficult (if required, depends on intended use)	Primary	Not required	-	-
	ILV	Not required	-	-
	Confirmatory (if required)	-	-	-

For any special comments or remarkable points concerning the analytical methods for the determination of residues in plant matrices, please refer to Appendix 2.

Table 5.3-12: Statement on extraction efficiency

	Method for products of plant origin
Required, available from:	A cross-validation study on plant matrices has been performed; please refer to KCP 5.2/16 (study VAL25/21).

For the detailed evaluation of (additional) studies on extraction efficiency, it is referred to Appendix 2.

zRMS comments:
The SAP2101F product is intended to be used in cereals (wheat and barley). Sufficient analytical methods for the determination of folpet (Sum of folpet and phtalamide, expressed as folpet) in plant matrices (all kinds of matrices) with appropriate LOQ are available.
The detailed of the methods are presented in Appendix 2.

Extraction efficiency:
A cross-validation study on plant matrices has been performed.
Wheat grain samples with incurred residues of folpet and metabolites were extracted with both extraction conditions, the one applied during the ^{14}C-metabolism studies and the extraction conditions of the method validated under the scope of LabRP GLP studies (VAL22/21), in order to evaluate the extraction efficiency.
The extraction efficiency was sufficiently proven since the difference between the two methods was lower than 30% for all analytes quantifiable. This is in accordance with requirements set on SANTE/2017/10632, Rev. 4, 23 February 2022.
The detailed of the study is presented in Appendix 2.

5.3.3.3 Description of analytical methods for the determination of residues in animal matrices (KCP 5.2)

An overview on the acceptable methods and possible data gaps for analysis of folpet in animal matrices is given in the following tables. For the detailed evaluation of new/additional studies it is referred to Appendix 2.

Table 5.3-13: Validated methods for food and feed of animal origin (if appropriate)

Component of residue definition: Phthalimide, expressed as folpet				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (*i.e.* GC-MS or HPLC-UV)**	**Author(s), year / missing**
Milk	Primary	0.01 mg/kg	LC-MS/MS	Wiesner, F., Breyer, N., Trümper, C. (2016) / new study under EU review Report no S16-00672 (BEL-1602V)
	ILV	0.01 mg/kg	LC-MS/MS	Mewis, A. (2016) / new study under EU review Report no S16-00717
	Confirmatory (if required)	0.01 mg/kg	LC-MS/MS	Wiesner, F., Breyer, N., Trümper, C. (2016) / new study under EU review Report no S16-00672 (BEL-1602V)
Eggs	Primary	0.01 mg/kg	LC-MS/MS	Schlewitz, P. (2015) / new study under EU review Report no R B4281 Wiesner, F., Breyer, N., Trümper, C. (2016) / new study under EU review Report no S16-00672 (BEL-1602V)
	ILV	0.01 mg/kg	LC-MS/MS	Mewis, A. (2016) / new study under EU review Report no S16-00717
	Confirmatory (if required)	0.01 mg/kg	LC-MS/MS	Schlewitz, P. (2015) / new study under EU review Report no R B4281
Muscle	Primary	0.01 mg/kg	LC-MS/MS	Schlewitz, P. (2015) / new study under EU review Report no R B4281
	ILV	0.01 mg/kg	LC-MS/MS	Meseguer (2016) / new study under EU review Report no S14-05780
	Confirmatory (if required)	0.01 mg/kg	LC-MS/MS	Schlewitz, P. (2015) / new study under EU review Report no R B4281
Fat	Primary	0.01 mg/kg 0.01 mg/kg	LC-MS/MS LC-MS/MS	Schlewitz, P. (2015) / new study under EU review Report no R B4281 Wiesner, F., Breyer, N., Trümper, C. (2016) / new study under EU review Report no S16-00672 (BEL-1602V)
	ILV	0.02 mg/kg	LC-MS/MS	Mewis, A. (2016) / new study under EU review Report no S16-00717
	Confirmatory (if required)	0.01 mg/kg	LC-MS/MS	Schlewitz, P. (2015) / new study under EU review Report no R B4281
Kidney, liver	Primary	0.01 mg/kg	LC-MS/MS	Schlewitz, P. (2015) / new study under EU review Report no R B4281
	ILV	0.01 mg/kg	LC-MS/MS	Meseguer (2016) / new study under EU review Report no S14-05780
	Confirmatory (if required)	0.01 mg/kg	LC-MS/MS	Schlewitz, P. (2015) / new study under EU review Report no R B4281

For any special comments or remarkable points concerning the analytical methods for the determination of residues in animal matrices, please refer to Appendix 2.

Table 5.3-14: Statement on extraction efficiency

	Method for products of animal origin
Required, available from:	Not required.
Not required, because:	A new study on poultry metabolism performed for Renewal shows residues <0.01 mg/kg in all animal matrices and eggs. Though no extraction efficiency is required. For ruminant matrices, the studies supporting Folpet renewal are the same presented in DAR. In consequence, no samples from animal matrices are available with incurred residues. A cross validation study is not possible to be performed. According to SANTE 2017/10632 it is not expected that new animal metabolism studies or new animal feeding studies should be set up only in order to evaluate aspects of analytical methods and extraction efficiency.

For the detailed evaluation of (additional) studies on extraction efficiency please refer to Appendix 2.

zRMS comments:
Sufficient analytical methods for the determination of folpet (phtalamide, expressed as folpet) in animal matrices with appropriate LOQ are available.
The detailed of additional analytical methods analysing residues in milk, eggs, muscle, fat, kidney, and liver are presented in Appendix 2.

Extraction efficiency:
Regarding extraction efficiency in animal matrices, we agree with above statement presented in Table 5.3-5.

5.3.3.4 Description of methods for the analysis of soil (KCP 5.2)

An overview on the acceptable methods and possible data gaps for analysis of folpet in soil is given in the following tables. For the detailed evaluation of new/additional studies it is referred to Appendix 2.

Table 5.3-15: Validated methods for soil (if appropriate)

Component of residue definition: Folpet			
Method type	**Method LOQ**	**Principle of method (*i.e.* GC-MS or HPLC-UV)**	**Author(s), year / missing**
Primary	0.01 mg/kg	LC-MS/MS	Schlewitz, P. (2015) / new study under EU review Report no R B4282
Confirmatory	0.01 mg/kg	LC-MS/MS	Schlewitz, P. (2015) / new study under EU review Report no R B4282

For any special comments or remarkable points concerning the analytical methods for soil please refer to Appendix 2.

zRMS comments:
Sufficient analytical method for the determination of folpet in soil with LOQ of 0.01 mg/kg is available.
The detailed of analytical method is presented in Appendix 2.

5.3.3.5 Description of methods for the analysis of water (KCP 5.2)

An overview on the acceptable methods and possible data gaps for analysis of folpet in surface and drinking water is given in the following tables. For the detailed valuation of new/additional studies it is referred to Appendix 2.

Table 5.3-16: Validated methods for water (if appropriate)

Component of residue definition: Folpet				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
Drinking water	Primary	0.1 µg/L	GC-MS	Aris, D. (2011) / new study under EU review Report no ZEF0005
	ILV	0.1 µg/L	GC-MS	Maas, X., Bendig, P. (2015) / new study under EU review Report no P 3812 G
	Confirmatory	0.1 µg/L	GC-MS	Aris, D. (2011) / new study under EU review Report no ZEF0005
Surface water	Primary	0.1 µg/L	GC-MS	Maas, X., Bendig, P. (2015) / new study under EU review Report no P 3812 G
	Confirmatory	0.1 µg/L	GC-MS	Maas, X., Bendig, P. (2015) / new study under EU review Report no P 3812 G

For any special comments or remarkable points concerning the analytical methods for water please refer to Appendix 2.

zRMS comments:
Sufficient analytical methods for the determination of folpet in drinking and surface water with LOQ of 0.1 µg/L is available.
The detailed of analytical methods are presented in Appendix 2.

5.3.3.6 Description of methods for the analysis of air (KCP 5.2)

An overview on the acceptable methods and possible data gaps for analysis of folpet in air is given in the following tables. For the detailed evaluation of new/additional studies please refer to Appendix 2.

Table 5.3-17: Validated methods for air (if appropriate)

Component of residue definition: Folpet			
Method type	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
Primary	10.8 µg/m^3	GC-MS	Aris, D. (2012) / new study under EU review Report no ZEF0006
Confirmatory	According to SANTE/2020/12830 rev. 2 *if the analytical dectection technique of the method matches that used in either soil or water, analytical methods and either of these methods demonstrate suitable confirmatory methods, no further confirmatory information is required for air methods.* Please see conclusion of KCP 5.2/14.		

For any special comments or remarkable points concerning the analytical methods for air it is referred to Appendix 2.

zRMS comments:
Sufficient analytical method for the determination of folpet in air with LOQ of 10.8 µg/m^3 is available.
The detailed of analytical method is presented in Appendix 2.

5.3.3.7 Description of methods for the analysis of body fluids and tissues (KCP 5.2)

An overview on the acceptable methods and possible data gaps for analysis of folpet in body fluids and tissues is given in the following table. For the detailed evaluation of new/additional studies it is referred to Appendix 2.

Table 5.3-18: Methods for body fluids and tissues (if appropriate)

Component of residue definition: ~~Not applicable~~ No residue definition for body fluids/tissue is set (RAR, 2018)			
Method type	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
Primary	0.05 mg/L (Phthalimide) for urine	LC-MS/MS	Wiesner&Breyer, (2016) / new study under EU review Report no S16-02058
	0.01 mg/kg (Phthalimide) for meat	LC-MS/MS	Schlewitz, P. (2015) / new study under EU review Report no R B4281
Confirmatory	0.05 mg/L (Phthalimide) for urine	LC-MS/MS	Wiesner&Breyer, (2016) / new study under EU review Report no S16-02058
	0.01 mg/kg (Phthalimide) for meat	LC-MS/MS	Schlewitz, P. (2015) / new study under EU review Report no R B4281

For any special comments or remarkable points concerning the analytical methods for body fluids and tissues please refer to Appendix 2.

zRMS Comment:
According to EFSA Journal 2009;297, 1-80 an analytical method for body fluids (blood) was not required since folpet is not classified as toxic or highly toxic. However, in Regulation (EU) No 283/2013 it is stated that "(…) *methods, with a full description, shall be submitted for the analysis in body fluids and tissues for the active substance and relevant metabolites*" and this is a requirement of SANTE/2020/12830. According to the SANTE/2020/12830: "*Analytical methods for monitoring residues in body fluids and tissues are required for detection of active substances and/or metabolites in humans and animals after possible intoxications or for biomonitoring purposes, regardless of their toxicological classification*."
Therefore, an analytical method for the residues of folpet in body fluids and tissues is required.

It should be noted that in RAR (2018) no residue definition for body fluids/tissue is set. The residue definition for in animal matrices currently includes phthalimide, expressed as folpet.
Analytical methods have been submitted under this application. The limit of quantification was established at 0.05 mg/L for phthalimide in urine and 0.01 mg/kg for phthalimide in meat.
According to SANTE/2020/12830 – rev.2, which is now in force, the LOQ shall be at 0.01 mg/L for body fluids.
Therefore, a data gap is proposed for a lower LOQ of 0.01 mg/L in accordance to the Guidance Document.
Any further data should be addressed at active substance level.
The detailed evaluation of the study is presented in Appendix 2.

5.3.3.8 Other studies/ information

Not relevant.

Appendix 1 Lists of data considered in support of the evaluation

List of data submitted by the applicant and relied on

Data point	Author(s)	Year	**Title** **Company Report No.** **Source (where different from company)** **GLP or GEP status** **Published or not**	Vertebrate study Y/N	Owner
KCP 5.1.1/01	Morais, F.	2022	PROTHIOCONAZOLE 120 g/L + FOLPET 300 g/L SC (SAP2101F): Physical, chemical and technical properties of the plant protection product Report no EF/371/21 – Interim Report (T0): Annex 1 – Prothioconazole and Folpet method validation and quantification ASCENZA Agro, S.A. GLP Unpublished	N	ASCENZA Agro, S.A.
KCP 5.1.1/02	Morais, F.	2022	PROTHIOCONAZOLE 120 g/L + FOLPET 300 g/L SC (SAP2101F): Physical, chemical and technical properties of the plant protection product Report no EF/371/21 – Interim Report (T0): Annex 3 – Prothioconazole-desthio and Toluene method validation and quantification ASCENZA Agro, S.A. GLP Unpublished	N	ASCENZA Agro, S.A.
KCP 5.1.1/03	Morais, F.	2022	PROTHIOCONAZOLE 120 g/L + FOLPET 300 g/L SC (SAP2101F): Physical, chemical and technical properties of the plant protection product Report no EF/371/21 – Interim Report (T0): Annex 4 – PMM method validation and quantification ASCENZA Agro, S.A. GLP Unpublished	N	ASCENZA Agro, S.A.
KCP 5.1.1/04	Morais, F.	2022	PROTHIOCONAZOLE 120 g/L + FOLPET 300 g/L SC (SAP2101F): Physical, chemical and technical properties of the plant protection product Report no EF/371/21 – Interim Report (T0): Annex 5 – CCl_4 method validation and quantification ASCENZA Agro, S.A. GLP Unpublished	N	ASCENZA Agro, S.A.
~~KCP 5.1.2/01~~	~~Stolze, J.~~	~~2022~~	~~Study on the Residue Behaviour of Prothioconazole in Oilseed Rape after Treatment with Prothioconazole 300 EC at six Sites under Field Conditions in Southern Europe, 2021~~ ~~Report no IF21-05707367~~ ~~SGS INSTITUT FRESENIUS GmbH~~ ~~GLP~~ ~~Unpublished~~	~~N~~	~~ASCENZA Agro, S.A.~~

Data point	Author(s)	Year	**Title** **Company Report No.** **Source (where different from company)** **GLP or GEP status** **Published or not**	Vertebrate study Y/N	Owner
KCP 5.1.2/02 (equivalent to KCP 10.2.1/01)	Schuler L.	2022	Analytical Summary: Analytical Method for the Determination of Prothioconazole and Folpet Eurofins Agroscience Services Study No S21-05200 GLP Unpublished	N	ASCENZA Agro, S.A.
KCP 5.1.2/03 (equivalent to KCP 10.2.1/02)	Schuler L.	2022	Analytical Summary: Analytical Method for the Determination of Prothioconazole and Folpet Eurofins Agroscience Services Study No S21-05199 GLP Unpublished	N	ASCENZA Agro, S.A.
KCP 5.1.2/04 (equivalent to KCP 10.6.2/01)	Lingott J.	2022	Analytical phase report: 'Prothioconazole + Folpet 120+300 g/L SC – SAP2101F': Effects on the Seedling Emergence and Growth of Six Non-Target Terrestrial Plant Species under Greenhouse Conditions Eurofins Agroscience Services Study No S21-05016-L2 GLP Unpublished	N	ASCENZA Agro, S.A.
KCP 5.1.2/05 (equivalent to KCP 10.6.2/02)	Lingott J.	2022	Analytical phase report: Prothioconazole + Folpet 120+300 g/L SC – SAP2101F': Effects on the Vegetative Vigour of Six Non-Target Terrestrial Plant Species under Greenhouse Conditions Eurofins Agroscience Services Study No S21-05017-L2 GLP Unpublished	N	ASCENZA Agro, S.A.
KCP 5.1.2/06 (equivalent to KCP 10.3.1.3/02)	Rastogi T.	2022	Analytical phase plan:SAP2101F: Honey Bee (*Apis mellifera* L.) Larval Toxicity Test following Repeated Exposure under laboratory conditions Eurofins Agroscience Services Study No.S21-05007-L3 GLP Unpublished	N	ASCENZA Agro, S.A.
KCP 5.1.2/07	Jooß, S.	2022	Validation of a Residue Analytical Method for the Determination of Folpet and its Metabolites in Cereal Matrices. Report No. S22-01156 Eurofins Agroscience Services. GLP Unpublished	N	ASCENZA Agro, S.A.
KCP 5.1.2/08	Jooß, S.	2022	Study on the Residue Behaviour of Folpet and its Metabolites in Processed Fractions of Barley after one Application of SAP 50SCF (Folpet 500 g/L, SC) in Norhtern Europe – 2021 Report No S22-04739 Eurofins Agroscience Services. GLP	N	ASCENZA Agro, S.A.

Data point	Author(s)	Year	**Title** **Company Report No.** **Source (where different from company)** **GLP or GEP status** **Published or not**	**Vertebrate study Y/N**	**Owner**
			Unpublished		
KCP 5.1.2/09 (equivalent to KCP 10.3.1.3/01)	Schreitmüller J.	2016	Analysis of Folpet in dosage solutions from Honey Bee Larvae Toxicity Innovative Environmental Services (IES) Ltd. Study No TRC14-245BA GLP Unpublished	N	Sapec Agro S.A and Belchim Crop Protection
KCP 5.1.2/10	Gordo, J	2022	Validation of the Analytical Method for the Determination of Folpet and Metabolites Residues in Wheat Report No. VAL22/21 Laboratório de Resíduos de Pesticidas ASCENZA AGRO, S.A. GLP Unpublished	N	ASCENZA Agro, S.A
KCP 5.2/01	Perny, A.	2015	Validation of the Analytical Method for the Determination of Folpet and Phthalimide in Grapes, Wine, Tomato, Cereal Grain and Sunflower Seeds Source: ANADIAG Report No.: R B4225 Date: 07/07/2015 GLP: yes Unpublished	N	Sapec Agro S.A. and ADAMA
KCP 5.2/02	Perny, A.	2015	Validation of the Analytical Method for the Determination of Folpet and Phthalimide in Grapes, Wine, Tomato, Cereal Grain and Sunflower Seeds – Amendment No. 1 Source: ANADIAG Report No.: R B4225 Date: 19/08/2015 GLP: yes Unpublished	N	Sapec Agro S.A. and ADAMA
KCP 5.2/03	Meseguer, C.	2015	Independent laboratory validation of the analytical method for the determination of folpet and phthalimide in crop matrices by LC-MS/MS Source: Eurofins Agroscience Services Chem SAS Report No.: S14-05779 Date: 24/03/2016 GLP: yes Unpublished	N	Sapec Agro S.A. and ADAMA
KCP 5.2/04	Wiesner, F., Breyer, N.	2016	Validation of the multi-residue method DFG-S19 for the determination of folpet and phthalimide in cereal grain and sunflower seeds	N	Sapec Agro S.A. and

Data point	Author(s)	Year	**Title** **Company Report No.** **Source (where different from company)** **GLP or GEP status** **Published or not**	Vertebrate study Y/N	Owner
			Source: Eurofins Agroscience Services Chem GmbH Report No.: S16-00559 (BEL-1601V) Date: 24/03/2016 GLP: yes Unpublished		ADAMA
KCP 5.2/05	Wiesner, F.	2016	Validation of the multi-residue method DFG-S19 for the determination of folpet and phthalimide in cereal grain and sunflower seeds – Amendment No. 1 Source: Eurofins Agroscience Services Chem GmbH Report No.: S16-00559 (BEL-1601V) Date: 29/04/2016 GLP: yes Unpublished	N	Sapec Agro S.A. and ADAMA
KCP 5.2/06	Hegmanns, C.	2016	Independent Laboratory Validation of the analytical method for the determination of folpet and phthalimide in cereal grain and sunflower seeds Source: Eurofins Agroscience Services EcoChem GmbH Report No.: S16-00716 Date: 02/05/2016 GLP: yes Unpublished	N	Sapec Agro S.A. and ADAMA
KCP 5.2/07	Wiesner, F., Breyer, N., Trümper, C.	2016	Validation of the multi-residue method DFG S19 for the determination of phthalimide in milk, fat and eggs Source: Eurofins Agroscience Services Chem GmbH Report No.: S16-00672 Date: 07/04/2016 GLP: yes Unpublished	N	Sapec Agro S.A. and ADAMA
KCP 5.2/08	Mewis, A.	2016	Independent Laboratory Validation of an analytical method for the determination of phthalimide in milk, eggs and fat Source: Eurofins Agroscience Services EcoChem GmbH Report No.: S16-00717 Date: 09/05/2016 GLP: yes Unpublished	N	Sapec Agro S.A. and ADAMA

Data point	Author(s)	Year	**Title** **Company Report No.** **Source (where different from company)** **GLP or GEP status** **Published or not**	Vertebrate study Y/N	Owner
KCP 5.2/09	Schlewitz, P.	2015	Validation of the analytical method for the determination of phthalimide, expressed as folpet, in milk, eggs, meat, fat and liver/kidney Source: ANADIAG Report No.: R B4281 Date: 09/09/2015 GLP: yes Unpublished	N	Sapec Agro S.A. and ADAMA
KCP 5.2/10	Meseguer, C.	2016	Independent Laboratory Validation of the analytical method for the determination of phthalimide in animal matrices by LC-MS/MS Source: Eurofins Agroscience Services Chem SAS Report No.: S14-05780 Date: 13/04/2016 GLP: yes Unpublished	N	Sapec Agro S.A. and ADAMA
KCP 5.2/11	Schlewitz, P.	2015b	Validation of the analytical method for the determination of folpet in soil Source: ANADIAG Report No.: R B4282 Date: 27/10/2015 GLP: yes Unpublished	N	Sapec Agro S.A. and ADAMA
KCP 5.2/12	Aris, D.	2011	Folpet and phthalimide: Validation of Methodology for the Determination of Residues of Folpet and Phthalimide in Drinking Water Source: Huntingdon Life Sciences, Ltd. Report No.: ZEF0005 Date: 25/10/2011 (Amendment No. 1: 17/02/2012) GLP: yes Unpublished	N	Sapec Agro S.A. and ADAMA
KCP 5.2/13	Maas, X., Bendig, P.	2015	Independent Laboratory Validation (ILV) of Analytical Methods for the Determination of Folpet and of Phthalimide in Water. Source: PTRL Europe Report No.: P 3812 G Date: 09/12/2015 GLP: yes Unpublished	N	Sapec Agro S.A. and ADAMA

Data point	Author(s)	Year	**Title** **Company Report No.** **Source (where different from company)** **GLP or GEP status** **Published or not**	**Vertebrate study Y/N**	**Owner**
KCP 5.2/14	Aris, D.	2012	Folpet and phthalimide: Validation of Methodology for the Determination of Residues of Folpet and Phthalimide in Air. Source: Huntingdon Life Sciences, Ltd. Report No.: ZEF0006 Date: 27/02/2012 GLP: yes Unpublished	N	Sapec Agro S.A. and ADAMA
KCP 5.2/15	Wiesner, F., Breyer, N.	2016	Validation of the multi-residue method DFG S19 for the determination of phthalimide in urine Source: Eurofins Agroscience Services Chem GmbH Report No.: S16-02058 Date: 17/04/2016 GLP: yes Unpublished	N	Sapec Agro S.A. and ADAMA
KCP 5.2/16	Gordo, J.	2023	Cross validation of an internal extraction method from LabRP vs. an Extraction Method Applied in ^{14}C-metabolism Studies for the Determination of Folpet and Metabolites in Wheat Report VAL 25/21 Laboratorio de Residuos de Pesticidas - ASCENZA AGRO, S.A. GLP Unpublished	N	ASCENZA Agro, S.A

List of data submitted or referred to by the applicant and relied on, but already evaluated at EU peer review

Data point	Author(s)	Year	**Title** **Company Report No.** **Source (where different from company)** **GLP or GEP status** **Published or not**	**Vertebrate study Y/N**	**Owner**
KCA 5.1.2	Heinemann	2001	Analytical determination of residues of JAU6476-sulfonic acid and JAU6476-desthio in/on cereals and canola by HPLC-MS/MS. Bayer AG Report No 00647 GLP: yes Unpublished	N	BAY
KCA 5.1.2	Billian P. and Druskus	2009	Residue analytical method 01132 for the determination of 1,2,4-triazole, trazole alanine, triazole acetic acid, triazole lactic acid in/on milk, egg, muscle, fat, liver and kidney by HPLC-MS/MS. Bayer CropScience AG, Report no 01132 GLP: yes	N	BAY

Data point	Author(s)	Year	**Title** **Company Report No.** **Source (where different from company)** **GLP or GEP status** **Published or not**	**Vertebrate study Y/N**	**Owner**
			Unpublished		
KCP 5.2	Weeren, R. D., Pelz, S.	2000	Modification M033 of method 00086: Validation of DFG method S 19 (extended revision) for the determination of residues of JAU 6476-desthio in materials of plant and animal origin. Dr. Specht & Partner, Chemische Laboratorien GmbH, Hamburg, Germany. Bayer AG, Report No.: 00086/M033, Date: 2000-11-20 GLP: Yes Unpublished	N	BAY
KCA 5.2	Class, Th.	2001	Independent laboratory validation of DFG method S19 (extended revision) for the determination of residues of JAU 6476-desthio (BAYER method 00086/M033) in plant materials. PTRL Europe, Ulm, Germany. Bayer AG, Report No.: P/B 484 G, Date: 2001-05-15 GLP: Yes Unpublished	N	BAY
KCA 5.2	Heinemann, O.	2000	Analytical determination of residues of JAU 6476 and desthio-JAU 6476 in/ on cereals by HPLC/MS/MS. Bayer AG, Report No.: 00598. Date: 2000-03-20 GLP: Yes Unpublished	N	BAY
KCA 5.1.2 KCA 5.2	Heinemann, O.	2001	Analytical determination of residues of JAU6476-3-hydroxy-desthio, JAU6476-4-hydroxy-desthio and JAU6476-desthio in/on matrices of animal origin by HPLC-MS/MS. Bayer AG, Report No.: 00655, Date: 2001-02-27 GLP: Yes Unpublished	N	BAY
KCA 5.1.2 KCA 5.2	Heinemann, O.	2001	Analytical determination of residues of JAU6476-3-hydroxy-desthio, JAU6476-4-hydroxy-desthio, and JAU6476-desthio in milk by HPLC-MS/MS (00655/M001). Bayer AG, Report No.: 00655/M001, Date: 2001-05-04 GLP: Yes Unpublished	N	BAY
KCA 5.2	Dubey, L.	2001	Independent laboratory validation of bayer methods 00655 and 00655/M001 for the determination of residues of JAU6476-3-hydroxy-desthio, JAU6476-4-hydroxy-desthio, JAU6476-desthio in/on matrices of animal origin by HPLC-MS/MS. Battelle, Geneva Research Centres, Carouge/Geneva, Switzerland. Bayer AG, Report No.: A-14-01-01, Date: 2001-10-16 GLP: Yes Unpublished	N	BAY

Data point	Author(s)	Year	**Title** **Company Report No.** **Source (where different from company)** **GLP or GEP status** **Published or not**	Vertebrate study Y/N	Owner
KCP 5.2	Schramel, O.	2000	Residue analytical method 00610 (MR-643/99) for the determination of JAU 6476 and the metabolites JAU6476-desthio and JAU6476-S-methyl in soil by HPLC-MS/MS. Bayer AG, Report No.: 00610, Date: 2000-07-13 GLP: Yes Unpublished	N	BAY
KCP 5.2	Steinhauer, S.	2001	Enforcement method 00086/M038 for the determination of the residues of JAU 6476-desthio in soil – validation of DFG method S 19 (extended revision) Dr. Specht & Partner, Chemische Laboratorien GmbH, Hamburg, Germany. Bayer AG, Report No.: 00086/M038, Date: 2001-07-25 GLP: Yes Unpublished	N	BAY
KCP 5.2	Sommer, H.	1999	Method for the determination of JAU 6476 and SXX 0665 in test water from aquatic toxicity tests by HPLC [Tox/Ecotox method] Bayer AG, Report No.: 00586, Date: 1999-05-28 GLP: Yes Unpublished	N	BAY
KCP 5.2	Sommer, H.	2001b	Enforcement method 00684 for determination of JAU 6476 and JAU 6476-desthio in drinking and surface water by HPLC-MS/MS. Bayer AG, Report No.: 00684, Date: 2001-10-23 GLP: Yes Unpublished	N	BAY
KCP 5.2	Maasfeld, W.	2002	Method for the determination of JAU 6476 in air by HPLC-MS/MS. Bayer AG, Report No.: 00724, Date: 2002-01-22 GLP: Yes Unpublished	N	BAY
KCP 5.2	Maasfeld, W.	2002	Method for the determination of JAU 6476-desthio (SXX 0665) in air by HPLC-MS/MS (Method-No. 00731) MR-003/02 ! 00731 ! P 605 00 6012 ! MO-02-002585 ! M-036729-01-1 GLP: Yes Unpublished	N	BAY

List of data submitted by the applicant and not relied on

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 5.1.2/01	Stolze, J.	2022	Study on the Residue Behaviour of Prothioconazole in Oilseed Rape after Treatment with Prothioconazole 300 EC at six Sites under Field Conditions in Southern Europe, 2021 Report no IF21-05707367 SGS INSTITUT FRESENIUS GmbH GLP Unpublished	N	ASCENZA Agro, S.A.

List of data relied on not submitted by the applicant but necessary for evaluation

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
-	-	-	-	-	-

Appendix 2 Detailed evaluation of submitted analytical methods

A 2.1 Analytical methods for prothioconazole

A 2.1.1 Methods used for the generation of pre-authorization data (KCP 5.1)

A 2.1.1.1.1 Analytical method 1

A 2.1.1.1.1.1 Method validation

Comments of zRMS:	The study has not been evaluated by zRMS-PL, because the product SAP2101F is not proposed to be used in rapeseed. This study is not required.

Reference:	KCP 5.2.1/01
Report	Study on the Residue Behaviour of Prothioconazole in Oilseed Rape after Treatment with Prothioconazole 300 EC at six Sites under Field Conditions in Southern Europe, 2021. Stolze, J. IF21-05707367
Guideline(s):	Yes Regulation (EC) No 1107/2009 of the European parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market and repealing council directives 79/117/EEC and 91/414/EEC. Commission Regulation (EU) No 283/2013 of 01 March 2013 implementing Regulation (EC) No 1107/2009 of the European Parliament and of the Council as regards the data requirements for active substances. SANTE/2020/12830, Rev.1 (24. February 2021)
Deviations:	No
GLP:	Yes
Acceptability:	~~Yes~~ Not evaluated

Materials and methods
1,2,4-T, TA, TAA and TLA were extracted with a mixture of methanol and water. An aliquot was filtered, concentrated and cleaned-up by dispersive C18-SPE step. The analytes were determined by LC-DMS/MS/MS, using two different HPLC stationary phases and an LCMS/MS instrument equipped with SelexION ion mobility technology which is based on planar differential spectrometry (DMS). Residues were quantified using stable isotopically labelled internal standards to compensate matrix effects.

The chromatographic conditions for the different metabolites were as follows:

Final Determination of 1,2,4-Triazole, Triazole alanine, Triazole acetic acid by LC-DMS/MS/MS

LC Systems	**Shimadzu MS 9**
Degasser:	DGU-20 A5R
Pump:	Nexera LC-30 AD
Injection System:	Nexera SIL-30 ACMP
Column oven:	CTO-20 AC
Control module:	CBM-20A Interface
LC-Conditions	
Guard Column:	Aquasil C18 125 Å
length:	10 mm
interior diameter:	3 mm
particle size:	3 µm

Manufacturer:	Thermo Scientific
Analytical Column:	Aquasil C18 125 Å
length:	150 mm
interior diameter:	3 mm
particle size:	3 µm
Manufacturer:	Thermo Scientific
Temperature of column thermostat:	60 °C
Injection volume:	3µl
Temperature of sample thermostat:	15 °C
Mobile Phase A:	ultra pure water/formic acid, 1000/5, (v/v)
Mobile Phase B:	methanol (LCMS grade)/formic acid, 1000/5, (v/v)

Gradient time table:

Time	Mobile Phase A	Mobile Phase B	Flow
[min]	[%]	[%]	[mL/min]
0.00	95	5	0.6
1.00	95	5	
1.30	60	40	
3.00	60	40	
3.10	5	95	
5.00	5	95	
5.51	95	5	
8.50	95	5	

Retention time:	1,2,4-Triazole ~ 1.6 minutes Triazole alanine ~ 1.3 minutes Traizole acetic acid~ 1.7 minutes
MS/MS Systems	AB Sciex, API 6500+ Triple Quad
Vacuum pump:	Varian / Agilent MS40+
Data system:	AB Sciex, Analyst, version 1.7.2

MS/MS Conditions

		1,2,4-Triazole	1,2,4-Triazole ISTD
Scan type:		MRM	
Ionisation mode:		ESI +	
SelexION (DMS):		On	
Curtain gas:	[psi]	35	
Collision gas:	[psi]	8	
DMS-Temperature	[°C]	Low 150	
DMS Resolution Enhancement:		Off	
Ionisation voltage:	[V]	2500	
Temperature:	[°C]	600	
Declustering potential:	[V]	101	
Collision energy:	[V]	27	
Collision cell exit potential:	[V]	8	
Compensation Voltage:	[V]	-14.3	
Separation Voltage:	[V]	3150	
DMS-Offset:	[V]	0	
Dwell time:	[msec]	30	
Transition used for evaluation:	[m/z]	70 → 43#	75 → 46

mass transition used for quantification

		Triazole alanine		TA ISTD
Scan type:		MRM		
Ionisation mode:		ESI +		
SelexION (DMS):		On		
Curtain gas:	[psi]	35		
Collision gas:	[psi]	8		
DMS-Temperature	[°C]	Low 150		
DMS Resolution Enhancement:		Off		
Ionisation voltage:	[V]	2500		
Temperature:	[°C]	600		
Declustering potential:	[V]	61	56	61
Collision energy:	[V]	19	17	19
Collision cell exit potential:	[V]	8	10	8
Compensation Voltage:	[V]	1		
Separation Voltage:	[V]	3150		
DMS-Offset:	[V]	-6		
Dwell time:	[msec]	50	30	50
Transition used for evaluation:	[m/z]	157 → 70#	157 → 88	162 → 75

mass transition used for quantification

MS/MS Conditions (continued)

		Triazole acetic acid		TAA ISTD
Scan type:		MRM		
Ionisation mode:		ESI +		
SelexION (DMS):		On		
Curtain gas:	[psi]	35		
Collision gas:	[psi]	8		
DMS-Temperature	[°C]	Low 150		
DMS Resolution Enhancement:		Off		
Ionisation voltage:	[V]	2500		
Temperature:	[°C]	600		
Declustering potential:	[V]	91		
Collision energy:	[V]	25	51	25
Collision cell exit potential:	[V]	10	8	10
Compensation Voltage:	[V]	-3		
Separation Voltage:	[V]	3150		
DMS-Offset:	[V]	-15		
Dwell time:	[msec]	30		
Transition used for evaluation:	[m/z]	128 → 70#	128 → 43	133 → 75

mass transition used for quantification

Final Determination of Triazole acetic acid (only straw matrix) and Triazole lactic acid by LC-DMS/MS/MS

HPLC System

LC Systems	**Shimadzu (MS 14/LCMS 9)**
Degasser:	DGU-20 A5R
Pump:	Nexera LC-30 AD
Injection System:	Nexera SIL-30 ACMP
Column oven:	CTO-20 AC
Control module:	CBM-20A Interface

LC-Conditions	
Guard Column:	Hypercarb
length:	10 mm
interior diameter:	4 mm
particle size:	5 µm
Manufacturer:	Thermo Scientific
Analytical Column:	Hypercarb
length:	100 mm
interior diameter:	4.6 mm
particle size:	5 µm
Manufacturer:	Thermo Scientific
Temperature of column thermostat:	30 °C
Injection volume:	5 µL
Temperature of sample thermostat:	15 °C
Mobile Phase A:	ultra pure water/formic acid, 1000/5, (v/v)
Mobile Phase B:	methanol (LCMS grade)/formic acid, 1000/5, (v/v)

Gradient time table:

Time	Mobile Phase A	Mobile Phase B	Flow
[min]	[%]	[%]	[mL/min]
0.0	100	0	0.8
3.0	100	0	
5.0	50	50	
9.0	50	50	
9.01	100	0	
12.5	100	0	

Retention time: Triazole acetic acid~ 8.3 minutes
Triazole lactic acid ~ 8.1 minutes

MS/MS Systems	**AB Sciex, API 6500+ Triple Quad**
Vacuum pump:	Varian / Agilent MS40+
Data system:	AB Sciex, Analyst, version 1.7.1

MS/MS Conditions

		Triazole acetic acid		TAA ISTD
Scan type:		MRM		
Ionisation mode:		ESI +		
SelexION (DMS):		On		
Curtain gas:	[psi]	25		
Collision gas:	[psi]	9		
DMS-Temperature	[°C]	Low 150		
DMS Resolution Enhancement:		Off		
Ionisation voltage:	[V]	2500		
Temperature:	[°C]	600		
Declustering potential:	[V]	91		
Collision energy:	[V]	25	51	25
Collision cell exit potential:	[V]	10	8	10
Compensation Voltage:	[V]	-3		
Separation Voltage:	[V]	3150		
DMS-Offset:	[V]	-15		
Dwell time:	[msec]	30		
Transition used for evaluation:	[m/z]	128 → 70#	128 → 43	133 → 75

		Triazole lactic acid		TLA ISTD
Scan type:		MRM		
Ionisation mode:		ESI +		
SelexION (DMS):		On		
Curtain gas:	[psi]	25		
Collision gas:	[psi]	9		
DMS-Temperature	[°C]	Low 150		
DMS Resolution Enhancement		Off		
Ionisation voltage:	[V]	2500		
Temperature:	[°C]	600		
Declustering potential:	[V]	91		
Collision energy:	[V]	25	21	25
Collision cell exit potential:	[V]	10	14	10
Compensation Voltage:	[V]	-0.80		
Separation Voltage:	[V]	3150		
DMS-Offset:	[V]	-20		
Dwell time:	[msec]	50		
Transition used for evaluation:	[m/z]	158 → 70#	158 → 112	163 → 75

mass transition used for quantification

Results and discussions

Table A 1: Recovery results from method validation of TDMs using the analytical method

Matrix	Analyte	Fortification level (mg/kg) (*n* = x)	Mean recovery (%)	RSD (%)	Comments
Straw	TA	0.01 (5)	84.9	6.9	157->70
		0.1 (5)	91.4	5.7	
		Overall (10)	83.1	6.4	
		0.01 (5)	91.5	12	157->88
		0.1 (5)	81.2	7.9	
		Overall (10)	86.3	11	
	TAA	0.01 (5)	94.2	5.4	128->70

Matrix	Analyte	Fortification level (mg/kg) (*n* = x)	Mean recovery (%)	RSD (%)	Comments
		0.1 (5)	92.4	2.4	
		Overall (10)	93.3	4.1	
		0.01 (5)	99.9	4.7	128->43
		0.1 (5)	92.6	3.0	
		Overall (10)	96.2	5.5	
	TLA	0.01 (5)	95.0	6.1	128->70
		0.1 (5)	87.8	3.3	
		Overall (10)	91.4	6.3	
		0.01 (5)	89.1	16	128->43
		0.1 (5)	85.7	13	
		Overall (10)	87.4	14	
	1,2,4-T	0.01 (5)	90.8	5.2	70 -> 43 Analytical Column Aquasil C18
		0.1 (5)	88.1	4.2	
		Overall (10)	89.4	4.7	
		0.01 (5)	98.7	7.4	70 -> 43 Analytical Column Hypercarb
		0.1 (5)	100	5.9	
		Overall (10)	99.6	6.4	

Table A 2: Characteristics for the analytical method used for validation of prothioconazole residues in straw

	TA	TAA	TLA	1,2,4-T
Specificity	No significant interferences at the retention time of analyte in any of the blank matrix tested (< LOD). Quantifier mass transition m/z 157 -> 70 (evaluated and used for quanti-fication) Qualifier mass transition m/z 157 -> 88 (monitored for confirmation of peak identity but was not used for quantification)	No significant interferences at the retention time of analyte in any of the blank matrix tested (< LOD). Quantifier mass transition m/z 128 -> 70 (evaluated and used for quanti-fication) Qualifier mass transition m/z 128 -> 43 (monitored for confirmation of peak identity but was not used for quantification)	No significant interferences at the retention time of analyte in any of the blank matrix tested (< LOD). Quantifier mass transition m/z 128 -> 70 (evaluated and used for quanti-fication) Qualifier mass transition m/z 128 -> 43 (monitored for confirmation of peak identity but was not used for quantification)	No significant interferences at the retention time of analyte in any of the blank matrix tested (< LOD). Since only one mass transition is vailable for 1,2,4-Triazole (1,24-T) validation sets for this analyte were performed on two chemicaly different stationary phases. Quantifier mass transition m/z 70 -> 43 Qualifier mass transition m/z 70 -> 43
Calibration (type, number of data points)	y = a + bx (with a = -0.04391 and b = 0.9990) R = 0.99989 Linear regression with 1/x weighting was performed. N=8	y = a + bx (with a = -0.0381 and b = 0.9741) R = 0.99997 Linear regression with 1/x weighting was performed. N=8	y = a + bx (with a = -0.01549 and b = 1.070) R = 0.99992 Linear regression with 1/x weighting was performed. N=8	y = a + bx (with a = -0.04239 and b = 0.9864) R = 0.99981 Linear regression with 1/x weighting was performed. N=8
Calibration range	The linearity of the detector response was confirmed by solvent standard solutions with the nominal working range 1.25 to 100 ng/mL (corresponding to 0.003 - 0.020 mg/kg).			

	TA	TAA	TLA	1,2,4-T
Specificity	No significant interferences at the retention time of analyte in any of the blank matrix tested (< LOD). Quantifier mass transition m/z 157 -> 70 (evaluated and used for quanti-fication) Qualifier mass transition m/z 157 -> 88 (monitored for confirmation of peak identity but was not used for quantification)	No significant interferences at the retention time of analyte in any of the blank matrix tested (< LOD). Quantifier mass transition m/z 128 -> 70 (evaluated and used for quanti-fication) Qualifier mass transition m/z 128 -> 43 (monitored for confirmation of peak identity but was not used for quantification)	No significant interferences at the retention time of analyte in any of the blank matrix tested (< LOD). Quantifier mass transition m/z 128 -> 70 (evaluated and used for quanti-fication) Qualifier mass transition m/z 128 -> 43 (monitored for confirmation of peak identity but was not used for quantification)	No significant interferences at the retention time of analyte in any of the blank matrix tested (< LOD). Since only one mass transition is vailable for 1,2,4-Triazole (1,24-T) validation sets for this analyte were performed on two chemicaly different stationary phases. Quantifier mass transition m/z 70 -> 43 Qualifier mass transition m/z 70 -> 43
Assessment of matrix effects is presented	No matrix effects were tested. The use of internal standards compensate for any matrix effects.			
Limit of determination/quantification	LOQ: 0.01 mg/kg LOD: 0.003 mg/kg (30% of the LOQ)			

Conclusion
The validity criteria for the analytical method have been met. The method is fit for purpose.

A 2.1.1.1.2 Analytical method 2

A 2.1.1.1.2.1 Method validation

Comments of zRMS:	Analytical methods for the determination of prothioconazole and folpet in test medium were validated with regard to recovery, linearity of detector response, repeatability, specificity, matrix effect, stability of working solutions, limit of quantification and limit of detection. LOQ: 0.0854 mg test item /L (0.00849 mg prothioconazole/L) 0.0854 mg test item /L (0.0226 mg folpet/L) The analytical methods fulfil the requirements of guideline SANTE/2020/12830 rev. 1. The method is acceptable.

Reference:	KCP 5.1.2/02
Report	Prothioconazole + Folpet 120 + 300 g/L SC: Toxicity to the Water Flea *Daphnia magna* Straus under Laboratory Conditions (Acute Immobilisation Test – Semi-Static). Schuler L., 2022, Study No. S21-05200.
Guideline(s):	SANTE/2020/12830 rev. 1/ 24/02/2021
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods
The content determination was performed in specimens following SAP2101F application. The technique applied for Prothioconazole and Folpet content determination was HPLC-MS/MS.

Chromatographic Conditions for Prothioconazole

HPLC system	Agilent 1290 Infinity HPLC system					
Column	Phenomenex Synergi™ Fusion-RP 80A, 50 mm x 2 mm i.d., 4 µm mean particle size (No. 00B-4424-B0) with 4 mm Fusion RP guard column (No. AJ0-7556, Phenomenex)					
Column oven temperature	30 °C					
Injection volume	30 µL					
Mobile phases	Eluent A: Water Eluent B: Acetonitrile					
Gradient	Time [min]	% Eluent A	% Eluent B	Flow [µL/min]		
	0.0	70	30	600		
	1.0	70	30	600		
	3.5	1	99	600		
	4.5	1	99	600		
	4.6	70	30	600		
	6.0	70	30	600		
Divert valve	0.5 min to 5.0 min to MS					
Retention time(s)	approx. 4.0 min for prothioconazole					
Mass Spectrometric Conditions for Prothioconazole						
MS system	SCIEX API 6500					
Ionisation type	Electrospray ionization (ESI)					
Polarity	Positive ion mode					
Scan type	MS/MS, Multiple Reaction Monitoring (MRM)					
Capillary voltage (IS)	5500 V (pos)		Ionspray turbo heater (TEM)	400 °C		
Curtain gas (CUR)	30 (arbitrary units)		Gas flow 1 (GS1)	60 (arbitrary units)		
Collision gas (CAD)	9 (arbitrary units)		Gas flow 2 (GS2)	60 (arbitrary units)		
Analyte monitored	Ion mass transition monitored [m/z]	Declustering potential (DP) [V]	Entrance potential (EP) [V]	Collision energy (CE) [V]	Cell exit potential (CXP) [V]	Dwell time [ms]
Prothioconazole	344 189*	11	10	37	10	100
	344 154	11	10	37	10	100

* used as quantifier

Chromatographic Conditions for Folpet						
HPLC system	Agilent 1290 Infinity HPLC system					
Column	Phenomenex Synergi™ Fusion-RP 80A, 50 mm x 2 mm i.d., 4 µm mean particle size (No. 00B-4424-B0) with 4 mm Fusion RP guard column (No. AJ0-7556, Phenomenex)					
Column oven temperature	30 °C					
Injection volume	80 µL					
Mobile phases	Eluent A: Water + 10 mM ammonium acetate + 0.1 % (v/v) formic acid Eluent B: Methanol + 0.1 % (v/v) formic acid					
Gradient	Time [min]	% Eluent A	% Eluent B	Flow [µL/min]		
	0.0	80	20	500		
	0.5	80	20	500		
	2.0	2	98	500		
	3.5	2	98	500		
	3.6	80	20	500		
	5.0	80	20	500		
Divert valve	0.5 min to 5.0 min to MS					
Retention time(s)	2.3 min for folpet					
Mass Spectrometric Conditions for Folpet						
MS system	SCIEX API 6500					
Ionisation type	Electrospray ionization (ESI)					
Polarity	Positive ion mode					
Scan type	MS/MS, Multiple Reaction Monitoring (MRM)					
Capillary voltage (IS)	5500 V (pos)		Ionspray turbo heater (TEM)	100 °C		
Curtain gas (CUR)	20 (arbitrary units)		Gas flow 1 (GS1)	50 (arbitrary units)		
Collision gas (CAD)	12 (arbitrary units)		Gas flow 2 (GS2)	30 (arbitrary units)		
Analyte monitored	Ion mass transition monitored [m/z]	Declustering potential (DP) [V]	Entrance potential (EP) [V]	Collision energy (CE) [V]	Cell exit potential (CXP) [V]	Dwell time [ms]

Folpet	313	130*	11	10	37	10	100
	315	130	11	10	37	10	100

* used as quantifier

Results and discussions

Table A 3: Recovery results from method validation of prothioconazole and folpet using the analytical method

Matrix	Analyte	Fortification level (mg t.i./L) (*n* = 5)	Mean recovery (%)	RSD (%)	Comments
Test medium	Prothioconazole	0.0854 (prothioconazole nominal 0.00849)	105	4	Overall mean recovery 107%; overall mean RSD 4%.
		26.0 (prothioconazole nominal 2.58)	109	4	
Test medium	Folpet	0.0854 (folpet nominal 0.0226)	110	5	Overall mean recovery 106.5%; overall mean RSD 8.5%.
		26.0 (folpet nominal 6.89)	103	12	

Table A 4: Characteristics for the analytical method used for validation of SAP2101F residues in test medium

	Prothioconazole	Folpet
Specificity	No significant interferences at the retention time of analyte in any of the blank matrix tested (< LOD). Quantifier mass transition *m/z* 344 ⟶ 189 (evaluated and used for quantification) Qualifier mass transition *m/z* 344 ⟶ 154 (monitored for confirmation of peak identity but was not used for quantification)	No significant interferences at the retention time of analyte in any of the blank matrix tested (< LOD). Quantifier mass transition *m/z* 313⟶130 (evaluated and used for quantification) Qualifier mass transition *m/z* 315⟶130 (monitored for confirmation of peak identity but was not used for quantification)
Calibration (type, number of data points)	Matrix matched R^2: 0.9974 Calibration curve: y = 1.84e+005 x + 6.8e+003 N=5	Matrix matched R^2: 0.9996 Calibration curve: y = 1.45e+004 x + -4.73e+003 N=5
Calibration range	0.1 − 10 ng/mL prothioconazole (corresponding to 0.00200 – 0.200 mg/L)	1.50 − 30 ng/mL folpet (corresponding to 0.00600 – 0.120 mg/L)
Assessment of matrix effects is presented	yes	yes
Limit of determination/quantification	LOD= 0.00200 mg prothioconazole /L LOQ = 0.00849 mg prothioconazole/L	LOD= 0.006 mg folpet/L LOQ = 0.0226 mg folpet/L

Conclusion

The validity criteria for the analytical method have been met.

A 2.1.1.1.3 Analytical method 3

A 2.1.1.1.3.1 Method validation

Comments of zRMS:	Analytical methods for the determination of prothioconazole and folpet in test medium were validated with regard to recovery, linearity of detector response, repeatability, specificity, matrix effect, stability of working solutions, limit of quantification and limit of detection. LOQ: 0.0954 mg test item /L (0.00948 mg prothioconazole/L) 0.0954 mg test item /L (0.0253 mg folpet/L) The analytical methods fulfil the requirements of guideline SANTE/2020/12830 rev. 1. The method is acceptable.

Reference:	KCP 5.1.2/03
Report	Prothioconazole + Folpet 120 + 300 g/L SC: Toxicity to the Single Cell Green Alga *Pseudokirchneriella subcapitata* Hindák under Laboratory Conditions. Schuler L, 2022, Study No. S21-05199
Guideline(s):	SANTE/2020/12830 rev. 1/ 24/02/2021
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

The content determination was performed in specimens following SAP2101F application. The technique applied for Prothioconazole and Folpet content determination was HPLC-MS/MS.

Chromatographic Conditions for Prothioconazole						
HPLC system	Agilent 1290 Infinity HPLC system					
Column	Phenomenex Synergi™ Fusion-RP 80A, 50 mm x 2 mm i.d., 4 µm mean particle size (No. 00B-4424-B0) with 4 mm Fusion RP guard column (No. AJ0-7556, Phenomenex)					
Column oven temperature	30 °C					
Injection volume	30 µL					
Mobile phases	Eluent A: Water Eluent B: Acetonitrile					
Gradient	Time [min]	% Eluent A	% Eluent B	Flow [µL/min]		
	0.0	70	30	600		
	1.0	70	30	600		
	3.5	1	99	600		
	4.5	1	99	600		
	4.6	70	30	600		
	6.0	70	30	600		
Divert valve	0.5 min to 5.0 min to MS					
Retention time(s)	approx. 2.8 min and 4.0 min respectively for prothioconazole					
Mass Spectrometric Conditions for Prothioconazole						
MS system	SCIEX API 6500					
Ionisation type	Electrospray ionization (ESI)					
Polarity	Positive ion mode					
Scan type	MS/MS, Multiple Reaction Monitoring (MRM)					
Capillary voltage (IS)	5500 V (pos)		Ionspray turbo heater (TEM)		400 °C	
Curtain gas (CUR)	30 (arbitrary units)		Gas flow 1 (GS1)		60 (arbitrary units)	
Collision gas (CAD)	9 (arbitrary units)		Gas flow 2 (GS2)		60 (arbitrary units)	
Analyte monitored	Ion mass transition monitored [m/z]	Declustering potential (DP) [V]	Entrance potential (EP) [V]	Collision energy (CE) [V]	Cell exit potential (CXP) [V]	Dwell time [ms]
Prothioconazole	344 189*	11	10	37	10	100
	344 154	11	10	37	10	100

*used as quantifier

Chromatographic Conditions for Folpet						
HPLC system	Agilent 1290 Infinity HPLC system					
Column	Phenomenex Synergi™ Fusion-RP 80A, 50 mm x 2 mm i.d., 4 µm mean particle size (No. 00B-4424-B0) with 4 mm Fusion RP guard column (No. AJ0-7556, Phenomenex)					
Column oven temperature	30 °C					
Injection volume	80 µL					
Mobile phases	Eluent A: Water + 10 mM ammonium acetate + 0.1 % (v/v) formic acid Eluent B: Methanol + 0.1 % (v/v) formic acid					
Gradient	Time [min]	% Eluent A	% Eluent B	Flow [µL/min]		
	0.0	80	20	500		
	0.5	80	20	500		
	2.0	2	98	500		
	3.5	2	98	500		
	3.6	80	20	500		
	5.0	80	20	500		
Divert valve	0.4 min to 4.0 min to MS					
Retention time(s)	approx. 2.2 min for folpet					
Mass Spectrometric Conditions for Folpet						
MS system	SCIEX API 6500					
Ionisation type	Electrospray ionization (ESI)					
Polarity	Positive ion mode					
Scan type	MS/MS, Multiple Reaction Monitoring (MRM)					
Capillary voltage (IS)	5500 V (pos)		Ionspray turbo heater (TEM)		100 °C	
Curtain gas (CUR)	20 (arbitrary units)		Gas flow 1 (GS1)		50 (arbitrary units)	
Collision gas (CAD)	12 (arbitrary units)		Gas flow 2 (GS2)		30 (arbitrary units)	
Analyte monitored	Ion mass transition monitored [m/z]	Declustering potential (DP) [V]	Entrance potential (EP) [V]	Collision energy (CE) [V]	Cell exit potential (CXP) [V]	Dwell time [ms]
Folpet	313 130*	11	10	37	10	100
	315 130	11	10	37	10	100

*used as quantifier

Results and discussions

Table A 5: Recovery results from method validation of prothioconazole and folpet using the analytical method

Matrix	Analyte	Fortification level (mg/L) (*n* = x)	Mean recovery (%)	RSD (%)	Comments
Prothioconazole					
Test item	0.00948 mg/L	0.0954	102	2	-
	12.9 mg/L	130	107	2	-
Folpet					
Test item	0.0253 mg/L	0.0954	105	11	-
	34.5 mg/L	130	81	12	-

Table A 6: Characteristics for the analytical method used for validation of prothioconazole and folpet residues in test medium

	Prothioconazole	**Folpet**
Specificity	No significant interferences at the retention time of analyte in any of the blank matrix tested (< LOD). Quantifier mass transition *m/z* 344 → 189 (evaluated and used for quantification)	No significant interferences at the retention time of analyte in any of the blank matrix tested (< LOD). Quantifier mass transition *m/z* 313 → 130 (evaluated and used for quantification)

	Prothioconazole	**Folpet**
	Qualifier mass transition *m/z* 344 → 154 (monitored for confirmation of peak identity but was not used for quantification)	Qualifier mass transition *m/z* 315 → 130 (monitored for confirmation of peak identity but was not used for quantification)
Calibration (type, number of data points)	Matrix matched, Coefficient of Correlation R^2:0.998 Calibration curve: y = 5.43e+004 x + 789 N=5	Matrix matched, Coefficient of Correlation R^2:0.9970 Calibration curve: y = y = 1.9e+004 x + -2.72e+004 N=5
Calibration range	0.1 − 10 ng/mL (corresponding to 0.00200 – 0.200 mg/L)	1.80 − 30 ng/mL (corresponding to 0.00720 – 0.120 mg/L)
Assessment of matrix effects is presented	yes	yes
Limit of determination/quantification	LOD = 0.00200 mg prothioconazole/L LOQ = 0.00948 mg prothioconazole/L	LOD = 0.00720 mg folpet/L LOQ = 0.0253 mg folpet/L

Conclusion
The validity criteria for the analytical method have been met.

A 2.1.1.1.4 Analytical method 4

A 2.1.1.1.4.1 Method validation

Comments of zRMS:	Analytical methods for the determination of prothioconazole and folpet in spray solution (tap water + 0.5 % formic acid) were validated with regard to recovery, linearity of detector response, repeatability, specificity, matrix effect, stability of working solutions, limit of quantification and limit of detection. LOQ: 1500 mg test item /L (149 mg prothioconazole/L) 1500 mg test item /L (398 mg folpet/L) The analytical methods fulfil the requirements of guideline SANTE/2020/12830. The method is acceptable.

Reference:	KCP 5.1.2/04
Report	Prothioconazole + Folpet 120+300 g/L SC – SAP2101F': Effects on the Seedling Emergence and Growth of Six Non-Target Terrestrial Plant Species under Greenhouse Conditions. Lingott J., (2022), Study No. S21-05016 Analytical Phase Code S21-05016-L2
Guideline(s):	Regulation (EC) No 283/2013 and 284/2013 implementing Regulation (EC) 1107/2009 of the European Parliament and of the Council of 21st of October 2009 concerning the Placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC. SANTE/2020/12830 rev. 1
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods
In brief, for prothioconazole and folpet samples in tap water + 0.5 % formic acid were diluted with methanol and if necessary, diluted further with methanol / ultra-pure water (1/1, v/v) + 0.5 % formic acid. Quantifica-

tion was performed by use of LC-MS/MS detection.

Chromatographic conditions for prothioconazole and folpet in tap water + 0.5 % formic acid						
HPLC system	liquid chromatograph system (Shimadzu, HPLC pump LC-30 AD, autosampler SIL-30ACMP)					
Pre-column	none					
Column	Ascentis Express C18, 50 x 2.1 mm, 2.7 µm, Supelco					
Column oven temperature	30 °C					
Injection volume	20 µL					
Mobile phases	Eluent A: ultra-pure water + 0.1 % formic acid + 5 mM ammonium formeate Eluent B: methanol + 0.1 % formic acid + 5 mM ammonium formeate					
Gradient	Time [min]	% Eluent A	% Eluent B	Flow [µL/min]		
	0.0	20	80	500		
	0.5	20	80	500		
	3.5	98	2	500		
	4.5	98	2	500		
	5.0	20	80	500		
Divert valve	Not used					
Retention time(s)	prothioconazole: approx. 3.4 min; folpet: approx. 3.1 min					
Mass spectrometric conditions for prothioconazole and folpet in tap water + 0.5 % formic acid						
MS system	SCIEX API 4000					
Ionisation type	Electrospray ionization (ESI)					
Polarity	Positive					
Scan type	MS/MS, Multiple Reaction Monitoring (MRM)					
Capillary voltage (IS)	5500 V (pos)		Ionspray turbo heater (TEM)		100 °C	
Curtain gas (CUR)	40 (arbitrary units)		Gas flow 1 (GS1)		50 (arbitrary units)	
Collision gas (CAD)	12 (arbitrary units)		Gas flow 2 (GS2)		30 (arbitrary units)	
Analyte monitored	Mass transition monitored *(m/z)*	Declustering potential (DP) [V]	Entrance potential (EP) [V]	Collision energy (CE) [eV]	Cell exit potential (CXP) [V]	Dwell time [ms]
prothioconazole	#	50	10	33	16	150
	344à152	50	10	95	18	150
folpet	#	11	10	37	10	200
	315à130	11	10	37	10	200

used for quantification. Both of the mass transitions listed can be used for quantification.

Results and discussions

The maximum storage interval of final sample extracts at typically 1 °C to 10 °C from first dilution until injection to the detection system was 7 days.

The limit of quantification (LOQ) was 1500 mg test item/L (149 mg/L for prothioconazole and 398 mg/L for folpet) with a limit of detection (LOD) of 20 mg/L for prothioconazole and 50 mg/L for folpet.

Table A 7: Recovery results from method validation of prothioconazole and folpet using the analytical method

prothioconazole (Validation)							
Matrix	**Fortification level (mg/L)**	**Recovery (%)**	**Mean Recovery (%)**	**Rel. Std. Dev. (%)**	**Replicates**	**Overall Mean Recovery (%)**	**Overall Rel. Std. Dev. (%)**
Mass Transition *m/z* (Used for Quantification)							
tap water + 0.5 % formic acid	149 (LOQ)	95, 100, 100, 91, 98	97	4	5	97	4
	1988	104, 94, 99, 92, 101	98	5	5		

No observable peak was detected in any control samples
Recoveries are without any blank correction

folpet (Validation)							
Matrix	**Fortification level (mg/L)**	**Recovery (%)**	**Mean Recovery (%)**	**Rel. Std. Dev. (%)**	**Replicates**	**Overall Mean Recovery (%)**	**Overall Rel. Std. Dev. (%)**
Mass Transition *m/z* (Used for Quantification)							
tap water + 0.5 % formic acid	398 (LOQ)	82, 86, 82, 78, 86	83	4	5	83	5
	5309	90, 80, 83, 79, 86	84	5	5		

No observable peak was detected in any control samples
Recoveries are without any blank correction

All mean recovery values for prothioconazole and folpet at all fortification levels for one (1) mass transition are within 70 % - 120 % with relative standard deviations ≤ 20 % and thereby comply with the standard acceptance criteria of the guidance document SANTE/2020/12830, rev. 1

Table A 8: Characteristics for the analytical method used for validation of prothioconazole and folpet residues in test medium

	Prothioconazole	**Folpet**
Specificity	Blank value < 30 % LOQ	Blank value < 30 % LOQ
Calibration (type, number of data points)	Matrix matched n= 5 Coefficient of Coeficient R:0.9996 Linear Regression Equation y = 5.58e+003 x + -1.01e+003	Matrix matched n= 5 Coefficient of Coeficient R:0.9990 Linear Regression Equation y = 2.2e+003 x + 506
Calibration range	2.50 ng/mL to 50 ng/mL	6.25 ng/mL to 125 ng/mL
Assessment of matrix effects is presented	yes	yes
Limit of quantification (samples analysed with dilution factor)	149 mg/L	398 mg/L

Conclusion
The method was successfully validated according to guidance document(s) SANTE/2020/12830, rev.1, (for risk assessment). The method is also compliant with all the requirements of SANTE/2020/12830, rev. 2.

A 2.1.1.1.5 Analytical method 5

A 2.1.1.1.5.1 Method validation

Comments of zRMS:	Analytical methods for the determination of prothioconazole and folpet in spray solution (tap water + 0.5 % formic acid) were validated with regard to recovery, linearity of detector response, repeatability, specificity, matrix effect, stability of working solutions, limit of quantification and limit of detection. LOQ: 1500 mg test item /L (149 mg prothioconazole/L) 1500 mg test item /L (398 mg folpet/L) The analytical methods fulfil the requirements of guideline SANTE/2020/12830. The method is acceptable.

Reference: KCP 5.1.2/05

Report: Prothioconazole + Folpet 120+300 g/L SC – SAP2101F: Effects on the Vegetative Vigour of Six Non-Target Terrestrial Plant Species under Greenhouse Conditions. Lingott J, (2022), Study No. S21-05017
Analytical Phase Code S21-05017-L2

Guideline(s): Regulation (EC) No 283/2013 and 284/2013 implementing Regulation (EC) 1107/2009 of the European Parliament and of the Council of 21st of October

2009 concerning the Placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC. SANTE/2020/12830 rev. 1

Deviations: No

GLP: Yes

Acceptability: Yes

Materials and methods

In brief, for prothioconazole and folpet samples of tap water + 0.5% formic acid were diluted with methanol and if necessary, diluted further with methanol / ultra-pure water (1/1, v/v) + 0.5% formic acid. Quantification was performed by use of LC-MS/MS detection.

Chromatographic conditions for prothioconazole and folpet in tap water + 0.5 % formic acid						
HPLC system	liquid chromatograph system (Shimadzu, HPLC pump LC-30 AD, autosampler SIL-30ACMP)					
Pre-column	none					
Column	Ascentis Express C18, 50 x 2.1 mm, 2.7 µm, Supelco					
Column oven temperature	30 °C					
Injection volume	20 µL					
Mobile phases	Eluent A: ultra-pure water + 0.1 % formic acid + 5 mM ammonium formeate Eluent B: methanol + 0.1 % formic acid + 5 mM ammonium formeate					
Gradient	Time [min]	% Eluent A	% Eluent B	Flow [µL/min]		
	0.0	20	80	500		
	0.5	20	80	500		
	3.5	98	2	500		
	4.5	98	2	500		
	5.0	20	80	500		
Divert valve	Not used					
Retention time(s)	prothioconazole: approx. 3.4 min; folpet: approx. 3.1 min					
Mass spectrometric conditions for prothioconazole and folpet in tap water + 0.5 % formic acid						
MS system	SCIEX API 4000					
Ionisation type	Electrospray ionization (ESI)					
Polarity	Positive					
Scan type	MS/MS, Multiple Reaction Monitoring (MRM)					
Capillary voltage (IS)	5500 V (pos)		Ionspray turbo heater (TEM)		100 °C	
Curtain gas (CUR)	40 (arbitrary units)		Gas flow 1 (GS1)		50 (arbitrary units)	
Collision gas (CAD)	12 (arbitrary units)		Gas flow 2 (GS2)		30 (arbitrary units)	
Analyte monitored	Mass transition monitored *(m/z)*	Declustering potential (DP) [V]	Entrance potential (EP) [V]	Collision energy (CE) [eV]	Cell exit potential (CXP) [V]	Dwell time [ms]
prothioconazole	313→130[#]	50	10	33	16	150
	344→152	50	10	95	18	150
folpet	313→130[#]	11	10	37	10	200
	315→130	11	10	37	10	200

[#] proposed (validation) and used (residue analysis, storage) for quantification. Both of the mass transitions listed can be used for quantification.

Results and discussions

The maximum storage interval of final sample extracts at typically 1 °C to 10 °C from first dilution until injection to the detection system was 7 days.

The limit of quantification (LOQ) was 1500 mg test item/L (149 mg/L for prothioconazole and 398 mg/L for folpet) with a limit of detection (LOD) of 20 mg/L for prothioconazole and 50 mg/L for folpet.

prothioconazole (Validation)							
Matrix	**Fortification level (mg/L)**	**Recovery (%)**	**Mean Recovery (%)**	**Rel. Std. Dev. (%)**	**Replicates**	**Overall Mean Recovery (%)**	**Overall Rel. Std. Dev. (%)**
Mass Transition *m/z* (Used for Quantification)							
tap water + 0.5 % formic acid	149 (LOQ)	101, 109, 109, 100, 110	106	5	5	104	4
	5467	104, 105, 102, 105, 97	103	3	5		

No observable peak was detected in any control samples
Recoveries are without any blank correction

folpet (Validation)							
Matrix	**Fortification level (mg/L)**	**Recovery (%)**	**Mean Recovery (%)**	**Rel. Std. Dev. (%)**	**Replicates**	**Overall Mean Recovery (%)**	**Overall Rel. Std. Dev. (%)**
Mass Transition *m/z* (Used for Quantification)							
tap water + 0.5 % formic acid	398 (LOQ)	89, 96, 93, 87, 93	92	4	5	88	7
	14601	92, 85, 89, 75, 85	85	8	5		

No observable peak was detected in any control samples
Recoveries are without any blank correction

Table A 9: Characteristics for the analytical method used for validation of prothioconazole and folpet residues in test medium

	Prothioconazole	**Folpet**
Specificity	Blank value < 30 % LOQ	Blank value < 30 % LOQ
Calibration (type, number of data points)	Matrix matched n= 5, Coefficient of correlation: 0.9990 Linear regression equation y = 3.87e+003 x + 3.3e+003	Matrix matched n= 5, Coefficient of correlation: 0.9990 Linear regression equation y = 1.53e+003 x + 2.76e+003
Calibration range	2.50 ng/mL to 100 ng/mL	6.25 ng/mL to 250 ng/mL
Assessment of matrix effects is presented	yes	yes
Limit of quantification (samples analysed with dilution factor)	149 mg/L	398 mg/L

Conclusion
The method was successfully validated according to guidance document(s) SANTE/2020/12830, rev.1, (for risk assessment). The method is also compliant with all the requirements of SANTE/2020/12830, rev. 2.

A 2.1.1.1.5.2 Method validation

Comments of zRMS:	The method was successfully validated for determination of Prothioconazole and Folpet in larval diet solutions with an LOQ of 0.994 mg/kg for Prothioconazole and 2.65 mg/kg for Folpet according to guidance document SANTE/2020/12830, rev.1. Remark: Missing data for folpet were completed by Evaluator. The method is acceptable.

Reference: KCP 5.1.2/06

Report: SAP2101F: Honey Bee (*Apis mellifera* L.) Larval Toxicity Test following Repeated Exposure under laboratory conditions, Rastogi T., (2022), Study No. S21-05007-L3

Guideline(s): Regulations (EU) 283/2013 and 284/2013 implementing Regulation (EC)

1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC
SANTE/2020/12830, rev.1

Deviations: No

GLP: Yes

Acceptability: Yes

Materials and methods

In brief, samples of larval diet were extracted with water containing 0.5% formic acid (10% of total volume) and acetone. The raw extracts were further diluted with acetonitrile containing 0.5% formic acid. The diluted samples were quantified by use of LC-MS/MS detection.

Chromatographic and Mass Spectrometric Conditions

A summary of the chromatographic and mass spectrometric conditions used for quantification is included in the following table:

Summary of chromatographic conditions for the determination of Prothioconazole and Folpet

Chromatographic conditions for Quantification of Prothioconazole and Folpet				
HPLC system	1290 Infinity Binary LC System, Agilent Technologies			
Pre-column	Phenomenex, C18, 4 x 3 mm			
Column	Agilent Zorbax Eclipse XDB-C18; 50 x 2.1 mm; 3.5 µm particle size (Prod. No.: 971700-902)			
Column oven temperature	40 °C			
Injection volume	10 µL			
Mobile phases	Eluent A: Water containing 5 mM ammonium formate and 0.1% of formic acid Eluent B: Methanol containing 5 mM ammonium formate and 0.5% of formic acid			
Gradient	Time [min]	% Eluent A	% Eluent B	Flow [µL/min]
	0.00	90.0	10.0	400
	1.00	90.0	10.0	400
	4.00	10.0	90.0	400
	6.00	10.0	90.0	400
	7.00	90.0	10.0	400
	8.00	90.0	10.0	400
Divert valve	Not used			
Retention time	Prothioconazole: approx. 4.7 min; Folpet: approx. 4.4 min			

Summary of mass spectrometric conditions for the determination of Prothioconazole and Folpet

Mass spectrometric conditions for Prothioconazole and Folpet			
MS system	Applied Biosystems API 5500 Q-Trap LC/MS System, SCIEX		
Ionisation type	Electrospray ionisation (ESI, TurboIonSpray)		
Polarity	Positive ion mode (pos)		
Scan type	Multiple Reaction Monitoring (MRM)		
Capillary voltage (IS)	4500 V (pos)	Ionspray turbo heater (TEM)	150 °C
Curtain gas (CUR)	Nitrogen set at 30 (arbitrary units)	Gas flow 1 (GS1)	Nitrogen set at 40 (arbitrary units)

Collision gas (CAD)	Nitrogen set at medium		Gas flow 2 (GS2)		Nitrogen set at 60 (arbitrary units)	
Analyte monitored	Mass transition monitored *(m/z)*	Declustering potential (DP) [V]	Entrance potential (EP) [V]	Collision energy (CE) [eV]	Cell exit potential (CXP) [V]	Dwell time [ms]
Prothioconazole	344 → 326#	56	10	15	28	50
	344 → 154	56	10	37	10	50
Folpet	313 → 130#	76	10	35	14	50
	313 → 260	76	10	17	22	50

Proposed (and/or used) for quantification but both of the mass transitions listed can be used for quantification.

Results and Discussion

Table A 10: Recovery results from method validation of prothioconazole and folpet using the analytical method

Prothioconazole (Full Validation)							
Matrix	**Fortification level**	**Recovery (%)**	**Mean Recovery (%)**	**Rel. Std. Dev. (%)**	**Replicates**	**Overall Mean Recovery (%)**	**Overall Rel. Std. Dev. (%)**
Mass Transition : 344 m/z → 326 m/z (Proposed for Quantification)							
Larval Diet	0.994 mg/kg (LOQ)	101, 108, 111, 105, 99.0	105	4.5	5	107	4.4
	9.94 mg/kg (10xLOQ)	108, 112, 107, 111, 113	110	2.1	5		
	Highest Level	99.5, 106	103	n.a.	2		
Mass Transition : 344 m/z → 154 m/z (Proposed for Confirmation)							
Larval Diet	0.994 mg/kg (LOQ)	91.8, 103, 102, 106, 97.4	100	5.7	5	102	4.5
	9.94 mg/kg (10xLOQ)	99.2, 105, 101, 102, 107	103	2.9	5		
	Highest Level	97.4, 108	102	n.a.	2		

n.a.: not applicable
Recoveries are without any blank correction
Analyte: Final determination as: Residues calculated as: Prothioconazole ~~Prothioconazole Prothioconazole~~

Folpet (Full Validation)							
Matrix	**Fortification level**	**Recovery (%)**	**Mean Recovery (%)**	**Rel. Std. Dev. (%)**	**Replicates**	**Overall Mean Recovery (%)**	**Overall Rel. Std. Dev. (%)**
Mass Transition : 313 m/z → 130 m/z (Proposed for Quantification)							
Larval Diet	2.65 mg/kg (LOQ)	93.4, 105, 105, 98.1, 110	102	6.2	5	104	6.2
	26.5 mg/kg (10xLOQ)	102, 109, 107, 100, 99.2	103	4.2	5		
	Highest Level	105, 118	112	n.a.	2		

Mass Transition : 313 m/z → 260 m/z (Proposed for Confirmation)							
Larval Diet	2.65 mg/kg (LOQ)	90.6, 104, 100, 102, 109	101	6.6	5	103	5.8
	26.5 mg/kg (10xLOQ)	102, 105, 101, 98.1, 104	102	2.	5		
	Highest Level	102, 116	109	n.a.	2		

Table A 11: Characteristics for the analytical method used for validation of prothioconazole and folpet residues in test medium

	Prothioconazole	Folpet
Specificity	Blank value < 30 % LOQ) Quantification Mass Transition 344 m/z → 326 m/z Confirmation Mass Transition 344 m/z → 154 m/z	Blank value < 30 % LOQ) Quantification Mass Transition 313 m/z → 130 m/z Confirmation Mass Transition 313 m/z → 260 m/z
Calibration (type, number of data points)	Matrix matched n= 7, Coefficient of correlation: 0.9988 Linear regression equation (344 m/z → 326 m/z) y = 2.08e+004 x + 6.88e+003 Coefficient of correlation: 0.9997 Linear regression equation (344 m/z → 154 m/z) y = 4.95e+003 x + - 611	Matrix matched n= 7, Coefficient of correlation: ≥0.9993 Linear regression equation (313 m/z → 130 m/z) y = 1.53e+003 x + 2.76e+003 Coefficient of correlation: ≥0.9997 Linear regression equation (313 m/z → 260 m/z) y = 1.64e+003 x + 477
Calibration range	0.497 ng/mL to 49.7 ng/mL	1.33 ng/mL to 133 ng/mL
Assessment of matrix effects is presented	yes	yes
Limit of determination/quantification	LOD= 0.199 mg/kg LOQ=0.994 mg/kg	LOD=0.532 mg/kg LOQ=2.65 mg/kg

Conclusion
The method was successfully validated for determination of Prothioconazole and Folpet in larval diet solutions with an LOQ of 0.994 mg/kg for Prothioconazole and 2.65 mg/kg for Folpet according to guidance document SANTE/2020/12830, rev.1. The method is also compliant with all the requirements of SANTE/2020/12830, rev. 2.

A 2.1.1.1.5.3 Method validation

Comments of zRMS:	The purpose of this study was the determination of the concentrations of Folpet in dose solutions from honeybee larvae toxicity study TRC14-245BA. The analytical method does not meet all SANTE/2020/12830, rev. 2 requirements for risk assessment methods. The presented validation data consist: - calibration data and calibration plot - chromatogram of a sample at level of 30.10 mg/L and 300.99 mg/L, chromatogram of blank sample), - recovery results from method validation of folpet (recovery sample spiked at one concentration of folpet (7359 mg Folpet/L) only) - no recovery sample spiked at the LOQ level. However, this method can be considered fit for purpose.

Reference: KCP 5.1.2/09

Report	Analysis of Folpet in dosage solutions from Honey Bee Larvae Toxicity, Schreitmüller J., Study No. TRC14-245BA
Guideline(s):	Not applicable
Deviations:	No
GLP:	Yes
Acceptability:	Fit for purpose

Materials and methods

An aliquot of 270 µL of the samples were diluted to 10 mL with acetonitrile containing 0.1 % phosphoric acid. The resulting solutions were analysed by HPLC with UV-detection.

HPLC Conditions

Autosampler:	Agilent 1260 HiP		
Pump:	Agilent 1260 Quarternary Pump		
Detector:	Agilent 1260 DAD		
Software:	Laura (Lab Logic)		
Column:	Kinetex C18 100 A; 50 mm x 4.6 mm; 2.6 µm		
Pre-column:	Phenomenex C18; 4 x 3 mm		
Eluent A:	Water with 0.1 % phosphoric acid		
Eluent B:	Acetonitrile with 0.1 % phosphoric acid		
Gradient:	Minutes	% Eluent A	% Eluent B
	0	90	10
	5	5	95
	8	5	95
	8.1	90	10
	13	90	10
Injection Volume:	5 µL		
Flow Rate:	2 mL/minute		
Temperature:	Room temperature		
Detection Wavelength:	280 nm		
Retention Time:	Approximately 3.8 minutes		

Results and Discussion

Concurrent with the sample analysis, a recovery sample spiked at a relevant concentration of Folpet (7359 mg Folpet/L) was prepared and analyzed in five-fold. The results obtained are presented in Table 2. A representative chromatogram is given in Figure 4.

The average recovery was found to be 104.5% of the spiked values with a relative standard deviation of 0.4 % Solvent without Folpet showed no interference at the retention time of Folpet. A representative chromatogram is given in Figure 3.

From the results of linearity and accuracy/precision, the system was considered to be sufficiently suitable for the purpose of this test.

Table A 12: Recovery results from method validation of folpet using the analytical method

Matrix	Analyte	Fortification level (mg/L) (*n* = 5)	Mean recovery (%)	RSD (%)	Comments
Test water	Folpet	7359	104.5	0.4	-

Table A 13: Characteristics for the analytical method used for validation of folpet residues in test medium

	Folpet
Specificity	HPLC-UV blank value < 30 % LOQ
Calibration (type, number of data points)	n= 5 Linear regression equation Y=1.1785x+0.7083

	Folpet
	R^2=0.9991
Calibration range	30.10 – 300.9 mg/L
Assessment of matrix effects is presented	yes
Limit of quantification	LOQ = 30.10 mg folpet/L

Conclusion

The results for the concentrations of Folpet in dosage solutions from honeybee larvae toxicity study TRC14-245BA show the correct preparation of the dosage solutions and the stability of the dosage solutions during the application. The analysis of the metabolite phthalimide was therefore not necessary.

A 2.1.2 Methods for post-authorization control and monitoring purposes (KCP 5.2)

A 2.1.2.1 Description of analytical methods for the determination of residues in plant matrices (KCP 5.2)

No new or additional studies have been submitted.

A 2.1.2.2 Description of analytical methods for the determination of residues in animal matrices (KCP 5.2)

No new or additional studies have been submitted.

A 2.1.2.3 Description of Methods for the Analysis of Soil (KCP 5.2)

No new or additional studies have been submitted.

A 2.1.2.4 Description of Methods for the Analysis of Water (KCP 5.2)

No new or additional studies have been submitted.

A 2.1.2.5 Description of Methods for the Analysis of Air (KCP 5.2)

No new or additional studies have been submitted.

A 2.1.2.6 Description of Methods for the Analysis of Body Fluids and Tissues (KCP 5.2)

No new or additional studies have been submitted.

A 2.1.2.7 A.2.A.9 Other Studies/ Information

No new or additional studies have been submitted.

A 2.2 Analytical methods for folpet

A 2.2.1 Methods used for the generation of pre-authorization data (KCP 5.1)

Please refer to methods used for the generation of pre-authorization data (KCP 5.1) inside of analytical methods used for prothioconazole and folpet.

A 2.2.1.1 Analytical method 1

Comments of zRMS:	The method was evaluated by zRMS-Greece in RR of SAP50SCF (December 2023). zRMS-Greece comments: *The LC-MS/MS analytical method has been fully validated in dry commodities (see KCP 5.1.2/01) for the determination of residues of folpet and metabolites and was found acceptable.* zRMS-PL comments: Below are some errors that the evaluator corrected. LOQ: 0.01 mg/kg for folpet in all matrices of wheat (wheat green material, grain and straw) 0.01 mg/kg for phthalimide in wheat (green material and grain) 0.05 mg/kg for phthalimide in wheat (straw) 0.05 mg/kg for phthalic acid in all matrices 0.05 mg/kg for phthalamic acid in all matrices The method was found to be valid according to the guidance document(s) SANTE/2020/12830, rev. 1 for risk assessment and/or monitoring and ENV/JM/MONO(2007)17.

Reference:	KCP 5.1.2/07
Report	Validation of a Residue Analytical Method for the Determination of Folpet and its Metabolites in Cereal Matrices, Jooß, S., 2022, Report No. S22-01156
Guideline(s):	Regulations (EU) 283/2013 and 284/2013 implementing Regulation (EC) 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC. SANTE/2020/12830, rev.1 (Guidance Document on Pesticide Analytical Methods for Risk Assessment and Post-approval Control and Monitoring Purposes). ENV/JM/MONO(2007)17 (OECD Guidance Document on Pesticide Residue Analytical Methods).
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods
The purpose of the analytical part of this study was to verify the concentration of the active ingredient of this test item in the test medium.

Quantification was performed by use of LC MS/MS with an isotopically labelled internal standard for Folpet, Phthalimide and Phthalic Acid. For Phthalamic Acid, quantification was performed by use of LC MS/MS with matrix-matched standards.

Chromatographic conditions for Folpet in Wheat (Grain)				
HPLC system	Agilent 1290 Infinity binary gradient pump, Agilent 1290 series column oven, CTC Analytics HTC PAL autosampler			
Pre-column	Phenomenex C_{18}, 4x3 mm, Art. No. AJO-8762			
Column	Supelco Ascentis Express C_{18} (100 mm x 2.1 mm, 2.7 µm, Serial No. USRB002316)			
Column oven temperature	40 °C			
Injection volume	10 µL			
Mobile phases	Eluent A: Water containing 20 mmol/L of ammonium formiate/Methanol (95/5, v/v) Eluent B: Methanol containing 20 mmol/L of ammonium formiate			
Gradient	Time [min]	% Eluent A	% Eluent B	Flow [µL/min]
	0.00	95	5	350
	3.00	10	90	350
	5.00	10	90	350
	5.10	95	5	350
	6.50	95	5	350
Divert valve	Not used			
Retention time	Folpet: approx. 3.9 min			

Chromatographic conditions for Folpet in Wheat (Green Material) and Wheat (Straw)				
HPLC system	Agilent 1290 Infinity binary gradient pump, Agilent 1290 series column oven, CTC Analytics HTC PAL autosampler			
Pre-column	Phenomenex C18, 4x3 mm, Art. No. AJO-8762)			
Column	Supelco Ascentis Express C18 (150 mm x 3.0 mm, 2.7 µm, Serial No. USRB003647)			
Column oven temperature	40 °C			
Injection volume	20 µL			
Mobile phases	Eluent A: Water containing 20 mmol/L of ammonium formate/Methanol (95/5, v/v)			
Eluent B: Methanol containing 20 mmol/L of ammonium formate Gradient				
	Time [min]	% Eluent A	% Eluent B	Flow [µL/min]
	0.0	95	5	350
	3.0	10	90	350
	7.0	10	90	350
	7.1	95	5	350
	8.5			
Divert valve	Not used			

Chromatographic conditions for Folpet in Wheat (Green Material) and Wheat (Straw)	
HPLC system	Agilent 1290 Infinity binary gradient pump, Agilent 1290 series column oven, CTC Analytics HTC PAL autosampler
Pre-column	Phenomenex C_{18}, 4x3 mm, Art. No. AJO-8762
Column	Phenomenex Synergi Polar RP 80Å (75 mm x 2.0 mm, 4.0 µm, Serial No. H18-089400)

Column oven temperature	40 °C			
Injection volume	50 µL			
Mobile phases	Eluent A: Water containing 0.5% of formic acid Eluent B: Methanol containing 0.5% of formic acid			
Gradient	Time [min]	% Eluent A	% Eluent B	Flow [µL/min]
	0.00	90	10	600
	2.00	90	10	600
	4.00	0	100	600
	6.00	0	100	600
	6.01	90	10	600
	8.00	90	10	600
Divert valve	Not used			
Retention time	Phthalimide: 3.6 approx. min			

Chromatographic conditions for Phthalimide in Wheat (Straw)				
HPLC system	Agilent 1290 Infinity binary gradient pump, Agilent 1290 series column oven, CTC Analytics HTC PAL autosampler			
Pre-column	Phenomenex C_{18}, 4x3 mm, Art. No. AJO-8762)			
Column	Phenomenex Synergi Polar RP 80Å (150 mm x 2.0 mm, 4.0 µm, Serial No. H22-130744)			
Column oven temperature	40 °C			
Injection volume	50 µL			
Mobile phases	Eluent A: Water containing 0.5% of formic acid Eluent B: Methanol containing 0.5% of formic acid			
Gradient	Time [min]	% Eluent A	% Eluent B	Flow [µL/min]
	0.00	95	5	600
	4.00	95	5	600
	12.00	0	100	600
	14.00	0	100	600
	14.01	95	5	600
	16.00	95	5	600
Divert valve	Not used			
Retention time	Phthalimide: 7.9 approx. min			

Chromatographic conditions for Phthalic Acid in all Matrices	
HPLC system	Agilent HPLC pump 1290 with degasser, HTC PAL autosampler, Agilent colum oven 1290 series
Pre-column	Phenomenex C18, 4 x 3 mmm
Column	Restek PFPP, Serial no. 16050248J (100 mm x 3.0 mm, 3.0 µm)
Column oven temperature	40 °C
Injection volume	10 µL
Mobile phases	Eluent A: Water containing 0.1 % formic acid (v/v) Eluent B: Methanol containing 0.1 % formic acid (v/v)

Gradient	Time [min]	% Eluent A	% Eluent B	Flow [µL/min]
	0.0	95	5	600
	2.0	95	5	600
	4.0	5	95	600
	6.0	5	95	600
	6.1	95	5	600
	8.0	95	5	600
Divert valve	Not used			
Retention time	Phthalic Acid: approx. 3.9 min			

Chromatographic conditions for Phthalamic Acid in all Matrices				
HPLC system	Agilent 1290 Infinity II Binary LC System, HTS-xt autosampler, MayLab MistraSwitch column oven			
Pre-column	Phenomenex C_{18} (4 x 3 mm)			
Column	Phenomenex Kinetex Biphenyl, Serial no. H20-176706 (100 mm x 4.6 mm, 2.6 µm)			
Column oven temperature	40 °C			
Injection volume	10 µL			
Mobile phases	Eluent A: Water containing 0.1 % formic acid (v/v) and 5mM of ammonium formate Eluent B: Methanol containing 0.1 % formic acid (v/v)			
Gradient	Time [min]	% Eluent A	% Eluent B	Flow [µL/min]
	0.00	90	10	500
	2.00	90	10	500
	6.00	5	95	500
	8.00	5	95	500
	8.01	90	10	500
	10.00	90	10	500
Divert valve	Not used			
Retention time	Phthalamic Acid: approx. 5.8 min			

Results and discussions

Matrix Effects

Folpet, Phthalimide, Phthalic Acid:

Isotopically labelled internal standard was used for quantification so that possible matrix effects on the detector response are compensated when using the response ratio of the analyte and the isotopically labelled internal standard for quantification. Therefore, matrix effects on detection were not determined within this study.

Phthalamic Acid:

Matrix enhancement was < 20 % for all investigated matrices and thus deemed to be insignificant for the quantitation transition. However, matrix-matched standards were used for quantification throughout the study.

Table A 1: Recovery results from method validation of folpet using the analytical method

Analyte	**Matrix**	**Fortification level (mg/L) (*n* = 5)**	**Mean recovery (%)**	**RSD (%)**	**Comments**
Folpet	Wheat (green	0.01	82.0	87.0	Mass Transition *m/z* 313

Analyte	Matrix	Fortification level (mg/L) (*n* = 5)	Mean recovery (%)	RSD (%)	Comments
	material)	0.1	91.9		=>130
	Wheat (grain)	0.01	95.7	93.5	
		0.1	91.3		
	Wheat (straw)	0.01	104	99.8	
		0.1	95.5		
	Wheat (green material)	0.01	86.4	89.2	Mass Transition *m/z* 315 =>130
		0.1	92.0		
	Wheat (grain)	0.01	91.8	91.2	
		0.1	90.6		
	Wheat (straw)	0.01	105	99.7	
		0.1	94.3		
Phthalimide	Wheat (green material)	0.01	96.8	95.2	Mass Transition *m/z* 313 =>130
		0.1	93.7		
	Wheat (grain)	0.01	96.4	94.1	
		0.1	91.8		
	Wheat (straw)	0.01	99.4	97.5	
		0.1	95.7		
	Wheat (green material)	0.01	105	98.3	Mass Transition *m/z* 315 =>130
		0.1	91.6		
	Wheat (grain)	0.01	103	97.4	
		0.1	91.5		
	Wheat (straw)	0.01	92.0	94.6	
		0.1	97.1		
Phthalic Acid	Wheat (green material)	0.01	92.1	94.1	Mass Transition *m/z* 313 =>130
		0.1	96.0		
	Wheat (grain)	0.01	86.2	87.2	
		0.1	88.3		
	Wheat (straw)	0.01	99.6	89.5	
		0.1	79.4		
	Wheat (green material)	0.01	101	100	Mass Transition *m/z* 315 =>130
		0.1	100		
	Wheat (grain)	0.01	84.5	83.2	
		0.1	82.0		
	Wheat (straw)	0.01	101	90.3	
		0.1	79.6		
Phthalamic Acid	Wheat (green material)	0.01	96.6	93.5	Mass Transition *m/z* 313 =>130
		0.1	90.4		
	Wheat (grain)	0.01	97.6	94.2	
		0.1	90.7		

Analyte	Matrix	Fortification level (mg/L) (*n* = 5)	Mean recovery (%)	RSD (%)	Comments
	Wheat (straw)	0.01	107	107	
		0.1	107		
	Wheat (green material)	0.01	95.2	92.2	Mass Transition *m/z* 315 =>130
		0.1	89.3		
	Wheat (grain)	0.01	94.9	92.8	
		0.1	90.7		
	Wheat (straw)	0.01	105	106	
		0.1	108		

Table A 2: Characteristics for the analytical method used for validation of folpet residues

	Folpet
Specificity	LC-MS/MS blank value < 30 % LOQ
Calibration (type, number of data points)	Correlation of peak area of different standard solutions with their corresponding concentrations, using a linear regression: Folpet grain(quantitation): R: 0.9995 Calibration curve: y = 1.0 x + 0.00265 number of data points = 8 Folpet grain(confirmation): R: 0.9988 Calibration curve: y = 1 x + 0.00558 number of data points = 8 Phthalimide grain/ green material(quantitation): R: 0.9999 Calibration curve: y = 2.44 x - 0.00129 number of data points = 8 Phthalimide grain/ green material(confirmation): R: 0.9996 Calibration curve: y = 0.813 x + 0.00115 number of data points = 8 Phthalic Acid (quantitation): R: 0.9992 Calibration curve: y = 0.474 x – 0.00558 number of data points = 8 Phthalic Acid (confirmation): R: 0.9997 Calibration curve: y = 1.14x – 0.0258 number of data points = 8 Phthalamic Acid grain (quantitation): R: 0.9999 Calibration curve: y = 3.16e+003 x + 6.95e+003 number of data points = 8 Phthalamic Acid grain (confirmation): R: 0.9998 Calibration curve: y = 2.38e+004 x + 3.11e+004 number of data points = 8

	Folpet
Calibration range	Folpet ~~0.003 to 0.30 mg reference item/L~~ 0.75 ng/mL to 75 ng/mL corresponding 0.003 mg/kg to 0.30 mg/kg Phthalimide ~~0.003 to 0.10 mg reference item/L~~ 0.75 ng/mL to 75 ng/mL for wheat (green material) and wheat (grain); 3.0 ng/mL to 100 ng/mL for wheat (straw) corresponding to 0.003 mg/kg to 0.30 mg/kg for wheat (green material and grain and 0.012 mg/kg to 0.4 mg/kg for wheat (straw) Phthalic Acid ~~0.015 to 1.5 mg reference item/L~~ 0.375 ng/mL to 375 ng/mL corresponding 0.015 mg/kg to 1.5 mg/kg Phthalamic Acid ~~0.015 to 0.85 mg reference item/L~~ 3.75 ng/mL to 200 ng/mL for wheat (green material) and wheat (grain) and from 1.88 ng/mL to 100 ng/mL for wheat (straw), corresponding to 0.015 mg/kg to 0.80 mg/kg
Assessment of matrix effects is presented	Yes
Limit of determination/quantification	~~LOQ = 0.01 mg/kg~~ ~~LOD = 0.003 mg/kg~~ LOQ = 0.01 mg/kg (folpet) LOQ = 0.01 mg/kg (phthalimide in wheat green material and wheat grain) LOQ = 0.05 mg/kg (phthalimide in wheat straw) LOQ = 0.05 mg/kg (Phthalic Acid) LOQ = 0.05 mg/kg (Phthalamic Acid) LOD = 0.003 mg/kg (folpet) LOD = 0.003 mg/kg (phthalimide in wheat green material and wheat grain) LOD = 0.012 mg/kg (phthalimide in wheat straw) LOD =0.015 mg/kg (Phthalic Acid) LOD =0.015 mg/kg (Phthalamic Acid)
Stability	An internal isotopically labelled standard was used for quantification and was added at the end of the sample extraction procedure. The internal standard is considered to show the same degradation behavior as the analyte itself so that the stability of the analyte in sample extracts was not investigated.

Table Conclusion
The methods were successfully validated for the determination of folpet, phthalimide, phthalic acid and phthalamic acid from the tested LOQs of 0.01 mg/kg and 0.05 mg/kg, respectively, up to 0.1 mg/kg or 0.5 mg/kg according to the guidance documents SANTE/2020/12830, rev. 1 for risk assessment and/or monitoring and ENV/JM/MONO(2007)17. The method is also compliant with all the requirements of SANTE/2020/12830, rev. 2.

A 2.2.1.2 Analytical method 2

Comments of zRMS:	The method was evaluated by zRMS-Greece in RR of SAP50SCF (December 2023). zRMS-Greece comments: *The LC-MS/MS analytical method has been fully validated in barley processed commodities (see KCP 5.1.2/03) for the determination of residues of folpet and metabolites and was found acceptable.* zRMS-PL comments: Below are some errors that the evaluator corrected. The method was successfully validated for determination of all analytes in brewer's yeast with an LOQ of 0.01 mg/kg for folpet and phthalimide and an LOQ of 0.05 mg/kg for

	phthalic acid and phthalamic acid according to guidance document(s) SANTE/2020/12830, rev.1. With regard to selectivity, accuracy and precision, the analytical methods were applied successfully for each analytical set when analysing the samples of the study.

Reference:	KCP 5.1.2/08
Report	Study on the Residue Behaviour of Folpet and its Metabolites in Processed Fractions of Barley after one Application of SAP50SCF (Folpet 500 g/L, SC) in Northern Europe- 2021, Jooß, S., 2022, Report No. S22-04739
Guideline(s):	Regulations (EU) 283/2013 and 284/2013 implementing Regulation (EC) 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC. SANTE/2020/12830, rev.1 (Guidance Document on Pesticide Analytical Methods for Risk Assessment and Post-approval Control and Monitoring Purposes). ENV/JM/MONO(2007)17 (OECD Guidance Document on Pesticide Residue Analytical Methods).
Deviations:	No
GLP:	Yes

Materials and methods

The purpose of this study was to verify the concentration of folpet and its metabolite in beer in comparison to its concentration in the RAC barley. The analytical method for beer analysis of folpet, phtalamide, phtalic and phtalamic acids was developed in this same study. The current summary of this study focusses on method validation for the sake of clarity, while in Section B7 the summary focusses in the residues obtained in the different processing matrices and in the RAC material for assessing the processing factors.

Extraction of Folpet from Processed Fractions of Barley

In brief, samples of barley grain, brewing malt, malt sprouts, dried brewers grain, brewer's yeast and beer were extracted with acetonitrile containing 1% of formic acid and water was added. Isotopically labelled internal standard was added to the raw extract before clean-up. Addition of internal standard amount must be adjusted depending on the residue level obtained within the samples if residues are higher.

Clean-up was carried out by partition into acetonitrile (addition of citrate salts, magnesium sulfate and sodium chloride) followed by dispersive SPE with PSA and magnesium sulfate. Quantification was performed by use of LC-MS/MS with an isotopically labelled internal standard.

The limit of quantification (LOQ) of the analytical method was 0.01 mg/kg for each matrix with a limit of detection (LOD) set at 0.003 mg/kg (defined as the lowest calibration standard, which is 30 % of the LOQ).

Extraction of Phthalimide from Processed Fractions of Barley

In brief, for phthalimide, samples of barley grain, brewing malt, malt sprouts, dried brewers grain, brewer's yeast and beer were extracted with acetonitrile containing 1% of formic acid and water was added. Isotopically labelled internal standard (addition of internal standard must be adjusted to the necessary dilution) was added to the raw extract before clean-up. Clean-up was carried out by partition into acetonitrile (addition of citrate salts, magnesium sulfate and sodium chloride) followed by concentration and dilution in water containing 0.1% of acetic acid. Quantification was performed by use of LC-MS/MS with an isotopically labelled internal standard.

The limit of quantification (LOQ) of the analytical method was 0.01 mg/kg for each matrix with a limit of detection (LOD) set at 0.003 mg/kg (defined as the lowest calibration standard, which is 30 % of the LOQ).

Extraction of Phthalic Acid from Processed Fractions of Barley

In brief, samples of barley grain, brewing malt, malt sprouts, dried brewer's grain, brewer's yeast and beer were extracted with acetonitrile containing 1% of formic acid and after addition of water. Isotopically labelled internal standard was added to the raw extract before clean-up. Addition of internal standard

amount must be adjusted depending on the residue level obtained within the samples if residues are higher. Clean-up was carried out by partition into acetonitrile (addition of magnesium sulfate and sodium chloride). Quantification was performed by use of LC-MS/MS with an isotopically labelled internal standard.
The limit of quantification (LOQ) of the analytical method was 0.05 mg/kg for each matrix with a limit of detection (LOD) set at 0.015 mg/kg (defined as the lowest calibration standard, which is 30 % of the LOQ).

Extraction of Phthalamic Acid from Processed Fractions of Barley
In brief, samples of barley grain, brewing malt, malt sprouts, dried brewers grain, brewer's yeast and beer were extracted with (water containing 0.1% of ammonium carbonate)/methanol (4/1, v/v). Clean-up was carried out by centrifugation and filtration using a syringe filter. Quantification was performed by use of LC-MS/MS with matrix-matched standards.
The limit of quantification (LOQ) of the analytical method was 0.05 mg/kg for each matrix with a limit of detection (LOD) set at 0.015 mg/kg (defined as the lowest calibration standard, which is 30 % of the LOQ).

Method Validation and Concurrent Recoveries
The analytical methods were previously validated at Eurofins Agroscience Services EAG Laboratories GmbH according to SANTE/2020/12830, rev. 1 for wheat (green material), wheat (grain) and wheat (straw) as representatives for dry matrices and matrices with high water content, respectively. Five (5) fortifications of untreated control samples at the level of LOQ and five (5) fortifications at the level of 10x LOQ were performed per analyte/matrix combination.
For each analytical set of sample analysis, the method's applicability in terms of accuracy and repeatability was assessed by concurrent recoveries.
For folpet, blank values of control sample materials used for recovery determinations did not exceed a level that would correspond to 30 % of the LOQ. Therefore, correction for blank values was not performed
For phthalimide, blank values of malt sprouts and dried brewers grain used for recovery determinations exceeded a level that would correspond to 30 % of the LOQ. Recoveries were corrected for apparent blank residues in this case. In the other matrices no residues of phthalimide > 30 % of LOQ were detected.
For phthalic acid, blank values of reagents and those control sample materials used for recovery determinations in all cases exceeded a level that would correspond to 30 % of the LOQ. Therefore, recoveries for phthalic acid were corrected for both, residues >30% of LOQ detected in control samples and residues >30% of LOQ detected in reagent blanks.
For phthalamic acid, blank values of barley grain, brewing malt and dried brewers grain used for recovery determinations exceeded a level that would correspond to 30 % of the LOQ. Recoveries were corrected for apparent blank residues in this case.
Fortifications for the individual analyte/matrix combinations were performed at levels of 0.01 mg/kg, 0.05 mg/kg, 0.1 mg/kg, 0.2 mg/kg, 0.5 mg/kg, 0.8 mg/kg, 2.0 mg/kg, 4.0 mg/kg and 5.0 mg/

Quantification was performed by use of LC MS/MS with an isotopically labelled internal standard for Folpet, Phthalimide and Phthalic Acid. For Phthalamic Acid, quantification was performed by use of LC MS/MS with matrix-matched standards.

Chromatographic conditions for Folpet in Malt Sprouts, Died Brewers Grain, Brewer's Yeast and Beer				
HPLC system	Agilent 1290 Infinity binary gradient pump, Agilent 1290 series column oven, CTC Analytics HTC PAL autosampler			
Pre-column	Phenomenex C18, 4x3 mm, Art. No. AJO-8762)			
Column	Supelco Ascentis Express C18 (150 mm x 3.0 mm, 2.7 µm, Serial No. USRB003647)			
Column oven temperature	40 °C			
Injection volume	20 µL			
Mobile phases	Eluent A: Water containing 20 mmol/L of ammonium formate/Methanol (95/5, v/v)			
Gradient	Time [min]	% Eluent A	% Eluent B	Flow [µL/min]
	0.0	95	5	350

	3.0	10	90	350
	7.0	10	90	350
	7.1	95	5	350
	8.5	95	5	350
Divert valve	Not used			
Retention time	Folpet: approx. 5.5 min			

Chromatographic conditions for Folpet in Barley (Grain) and Brewing Malt				
HPLC system	Agilent 1290 Infinity binary gradient pump, Agilent 1290 series column oven, CTC Analytics HTC PAL autosampler			
Pre-column	Phenomenex C18, 4x3 mm, Art. No. AJO-8762			
Column	Supelco Ascentis Express C_{18} (100 mm x 2.1 mm, 2.7 µm, Serial No. USRB002316)			
Column oven temperature	40 °C			
Injection volume	10 µL			
Mobile phases	Eluent A: Water containing 20 mmol/L of ammonium formiate/Methanol (95/5, v/v) Eluent B: Methanol containing 20 mmol/L of ammonium formiate			
Gradient	Time [min]	% Eluent A	% Eluent B	Flow [µL/min]
	0.00	95	5	350
	3.00	10	90	350
	5.00	10	90	350
	5.10	95	5	350
	6.50	95	5	350
	8.5			
Divert valve	Not used			
Retention time	Folpet: approx. 3.9 min			

Chromatographic conditions for Phthalimide in all Matrices				
HPLC system	Agilent 1290 Infinity binary gradient pump, Agilent 1290 series column oven, CTC Analytics HTC PAL autosampler			
Pre-column	Phenomenex C18, 4x3 mm, Art. No. AJO-8762			
Column	Phenomenex Synergi Polar RP 80Å (75 mm x 2.0 mm, 4.0 µm, Serial No. H18-089400)			
Column oven temperature	40 °C			
Injection volume	50 µL			
Mobile phases	Eluent A: Water containing 0.5% of formic acid Eluent B: Methanol containing 0.5% of formic acid			
Gradient	Time [min]	% Eluent A	% Eluent B	Flow [µL/min]
	0.00	90	10	600
	2.00	90	10	600
	4.00	0	100	600
	6.00	0	100	600
	6.01	90	10	600
	8.00	90	10	600
Divert valve	Not used			

Retention time	Phthalimide: 3.6 approx. min

Chromatographic conditions for Phthalic Acid in all Matrices				
HPLC system	Agilent HPLC pump 1290 with degasser, HTC PAL autosampler, Agilent colum oven 1290 series			
Pre-column	Phenomenex C18, 4 x 3 mmm			
Column	Restek PFPP, Serial no. 16050248J (100 mm x 3.0 mm, 3.0 µm)			
Column oven temperature	40 °C			
Injection volume	10 µL			
Mobile phases	Eluent A: Water containing 0.1 % formic acid (v/v) Eluent B: Methanol containing 0.1 % formic acid (v/v)			
Gradient	Time [min]	% Eluent A	% Eluent B	Flow [µL/min]
	0.0	95	5	600
	2.0	95	5	600
	4.0	5	95	600
	6.0	5	95	600
	6.1	95	5	600
	8.0	95	5	600
Divert valve	Not used			
Retention time	Phthalimide: 3.9 approx. min			

Results and discussions

Table A 1: Recovery results from method validation of folpet and its metabolites using the analytical method

~~Analyte~~	~~Matrix~~	~~n=x~~	~~Fortification level (mg/L)~~	~~Mean recovery (%)~~	~~RSD (%)~~	~~Comments~~
~~Folpet~~	~~Barley~~	~~5~~	~~0.01~~	~~85.5~~	~~5.5~~	~~Mass Transition *m/z* 315 =>130~~
			~~0.1~~	~~97.8~~		
	~~Brewing Malt~~	~~4~~	~~0.01~~	~~90.9~~	~~6.6~~	
			~~0.1~~	~~98.3~~		
	~~Malt sprouts~~	~~4~~	~~0.01~~	~~101~~	~~12~~	
			~~0.1~~	~~92.8~~		
	~~Dried Brewers Grain~~	~~4~~	~~0.01~~	~~95.7~~	~~6.0~~	
			~~0.1~~	~~92.9~~		
	~~Brewer's Yeast~~	~~6~~	~~0.01~~	~~91.2~~	~~11~~	
			~~0.1~~	~~81.5~~		
	~~Beer~~	~~4~~	~~0.01~~	~~93.4~~	~~5.5~~	
			~~0.1~~	~~99.2~~		

Phthalimide	Barley	5	0.01	106	9.05	Mass Transition *m/z* 148 =>102
			0.1	102		
	Brewing Malt	4	0.01	111	6.0	
			0.1	106		
	Malt sprouts	5	0.01	98.3	10	
			0.1	86.3		
	Dried Brewers Grain	4	0.01	102	7.9	
			0.1	93.3		
	Brewer's Yeast	6	0.01	106	9.9	
			0.1	90.9		
	Beer	4	0.01	88.4	8.0	
			0.1	94.8		
Phthalic Acid	Barley	5	0.01	94.6	5.1	Mass Transition *m/z* 165 =>77
			0.1	94.9		
	Brewing Malt	5	0.01	102	6.8	
			0.1	97.6		
	Malt sprouts	5	0.01	82.2	9.6	
			0.1	94.4		
	Dried Brewers Grain	4	0.01	89.2	7.7	
			0.1	92.7		
	Brewer's Yeast	6	0.01	94.3	6.7	
			0.1	100		
	Beer	4	0.01	108	3.2	
			0.1	103		
Phthalamic Acid	Barley	4	0.01	108	8.4	Mass Transition *m/z* 166 =>130
			0.1	101		
	Brewing Malt	4	0.01	99.3	9.9	
			0.1	105		
	Malt sprouts	5	0.01	97	8.2	
			0.1	106		
	Dried Brewers Grain	4	0.01	91.8	8.5	
			0.1	92.4		
	Brewer's Yeast	6	0.01	94.7	7.8	
			0.1	102		
	Beer	4	0.01	87.6	15	
			0.1	102		

Matrix	Fortification level (mg/kg)	Concurrent Recovery (%)	Mean Recovery (%)	Rel. Std. Dev. (%)	Replicates	Overall Mean Recovery (%)	Overall Rel. Std. Dev. (%)
Folpet (Mass Transition *m/z* 315→130 (Quantification))							
Barley Grain	0.01 (LOQ)	89.0, 78.8, 84.4, 74.8, 100	85.5	12	5	91.5	10
	0.1	94.4, 91.2, 99.2, 99.2, 105	97.8	5.5	5		
	0.80	94.5, 81.0, 97.5	91.0	9.7	3		
Brewing Malt	0.01 (LOQ)	96.8, 92.8, 81.2, 92.8	90.9	7.4	4	94.6	6.6
	0.1	98.0, 95.6, 103, 96.8	98.3	3.2	4		
Malt Sprout	0.01 (LOQ)	98.4, 106, 104, 96.0	101	4.6	4	93.0	12
	0.1	91.2, 93.6, 72.8, 82.0	84.9	11	4		
Dried Brewers Grain	0.01 (LOQ)	101, 95.2, 102, 85.2	95.7	7.9	4	94.3	6.0
	0.1	88.8, 91.2, 96.8, 94.8	92.9	3.9	4		
Brewer's Yeast	0.01 (LOQ)	100, 88.4, 86.4, 80.4, 90.4, 103	91.5	9.4	6	87.0	11
	0.1	78.4, 71.6, 75.2, 85.6, 93.6, 90.8	82.5	11	6		
Beer	0.01 (LOQ)	97.7, 96.7, 89.3, 89.9	93.4	4.7	4	96.3	5.2
	0.1	96.0, 105, 98.2, 97.4	99.2	4.0	4		

Matrix	Fortification level (mg/kg)	Concurrent Recovery (%)	Mean Recovery (%)	Rel. Std. Dev. (%)	Replicates	Overall Mean Recovery (%)	Overall Rel. Std. Dev. (%)
Phthalimide (Mass Transition *m/z* 148→102 (Quantification))							
Barley Grain	0.01 (LOQ)	100, 106, 106, 98.4, 119	106	7.7	5	104	5.8
	0.1	107, 102, 102, 94.0, 107	102	5.2	5		
	0.80	104, 101, 101	102	2.0	3		
Brewing Malt	0.01 (LOQ)	113, 104, 108, 117	111	4.9	4	106	6.0
	0.1	102, 102, 100, 99.6	101	1.5	4		
Malt Sprout	0.01 (LOQ)	90.0 (179), 89.2 (178), 100, 106, 106	98.3	8.5	5	92.8	10
	0.1	85.1 (94.0), 87.9 (96.8), 75.2, 81.6, 102	86.3	11	5		
	0.20	93.0, 94.4, 95.6	94.3	1.4			
Dried Brewers Grain	0.01 (LOQ)	110 (208), 108 (205), 98.4 (154), 91.2 (147)	102	8.7	4	97.6	7.9
	0.1	89.9 (99.6), 94.7 (104), 96.8 (102), 91.6 (97.2)	93.3	3.3	4		
Brewer's Yeast	0.01 (LOQ)	103, 100, 100, 106, 117, 120	108	8.0	6	105	6.3
	0.1	104, 100, 103, 99.6, 104, 103	102	2.0	6		
Beer	0.01 (LOQ)	100, 87.8, 77.9, 82.6	87.1	11	4	90.9	8.5
	0.1	97.5, 94.2, 90.6, 96.7	94.8	3.3	4		

Matrix	Fortification level (mg/kg)	Concurrent Recovery (%)	Mean Recovery (%)	Rel. Std. Dev. (%)	Replicates	Overall Mean Recovery (%)	Overall Rel. Std. Dev. (%)
Phthalic Acid (Mass Transition *m/z* 165→77 (Quantification))							
Barley Grain	0.05 (LOQ)	93.2 (235), 90.2 (232), 104 (186), 98.6 (181), 87.4 (216)	94.6	6.9	5	94.3	5.0
	0.5	93.0 (107), 94.0 (108), 98.6 (107), 96.2 (104), 92.7 (106)	94.9	2.6	5		
	2.0	89.3 (92.5)	-	-	1		
Brewing Malt	0.05 (LOQ)	105 (201), 107 (202), 89.4 (168), 95.2 (174), 112 (220)	102	9.1	5	99.0	6.7
	0.5	97.1 (107), 96.3 (106), 98.7 (107), 95.7 (104), 100 (111)	97.6	1.8	5		
	2.0	92.8 (95.5)	-	-	1		
Malt Sprout	0.05 (LOQ)	79.0 (329), 80.0 (330), 84.0 (230), 92.0 (238), 76.0 (362)	82.2	7.5	5	89.9	9.8
	0.5	91.8 (117), 95.6 (121), 93.4 (108), 94.8 (109), 96.4 (125)	94.4	1.9	5		
	4.0	105 (108)	-	-	1		
Dried Brewers Grain	0.05 (LOQ)	90.2 (227), 93.2 (230), 75.6 (252), 97.6 (274)	89.2	11	4	90.9	7.7
	0.5	98.7 (112), 91.3 (105), 90.6 (108), 90.2 (108)	92.7	4.4	4		
Brewer's Yeast	0.05 (LOQ)	105 (849), 96.0 (840), 108 (852), 94.6 (136), 106 (147), 103 (144)	102	5.4	6	102	3.7
	0.5	101 (175), 101 (176), 101 (176), 102 (106), 103 (107), 103 (107)	102	0.8	6		
Beer	0.05 (LOQ)	109 (152), 110 (153), 104 (147), 108 (151)	108	2.4	4	105	3.2
	0.5	101 (105), 102 (107), 104 (109), 102 (107)	103	1.3	4		

Matrix	Fortification level (mg/kg)	Concurrent Recovery (%)	Mean Recovery (%)	Rel. Std. Dev. (%)	Replicates	Overall Mean Recovery (%)	Overall Rel. Std. Dev. (%)
Phthalamic Acid (Mass Transition *m/z* 166→130 (Quantification))							
Barley Grain	0.05 (LOQ)	103 (234), 104 (235), 114 (220), 112 (218)	108	5.2	4	105	4.9
	0.5	102 (115), 101 (114), 101 (111), 100 (111)	101	0.81	4		
	5.0	103 (104)	-	-	1		
Brewing Malt	0.05 (LOQ)	94.1 (259), 90.8 (256), 106 (284), 107 (285)	99.3	8.1	4	102	9.6
	0.5	94.2 (111), 95.3 (112), 116 (133), 115 (133)	105	11	4		
Malt Sprout	0.05 (LOQ)	83.4, 105, 95.2, 92.0	94.0	9.7	4	99.0	8.4
	0.5	102, 101, 110, 109, 97.4	104	5.4	5		
	5.0	94.2	-	-	1		
Dried Brewers Grain	0.05 (LOQ)	91.8 (222), 90.1 (220), 95.1 (260), 90.1 (255)	91.8	2.6	4	96.5	8.5
	0.5	92.4 (105), 94.0 (107), 109 (125), 110 (126)	101	9.3	4		
Brewer's Yeast	0.05 (LOQ)	73.9, 77.2, 70.6, 97.0, 97.4, 98.6	85.8	15	6	93.7	13
	0.5	97.6, 98.0, 96.4, 107, 106, 105	102	4.7	6		
Beer	0.05 (LOQ)	76.3, 72.4, 97.2, 104	87.6	18	4	94.7	15
	0.5	95.8, 92.8, 108, 110	102	8.7	4		

Table A 2: Characteristics for the analytical method used for validation of folpet and metabolites residues

	Folpet – Phthalimide - Phthalic Acid - Phthalamic Acid
Specificity	LC-MS/MS blank value < 30 % LOQ
Calibration (type, number of data points)	Correlation of peak area of different standard solutions with their corresponding concentrations, using a linear regression: Folpet grain and brewing malt(quantitation): R: 0.9994 Calibration curve: y = 1 x + 0.00718 (r=0.9994) number of data points = 8 Folpet Malt Sprouts, Dried Brewers Grain and Brewer's Yeast (Quantitation): R: 0.9988 Calibration curve: y = 0.904 x + 0.00388 number of data points = 8 Folpet Beer (Quantitation): R: 0.9996 Calibration curve: y = 0.901 x + 0.00561 number of data points = 8 Phthalimide Barley (Grain) and Brewing Malt (quantitation): R: 0.9998 Calibration curve: y = 2.46 x + 0.0577 number of data points = 8

	Folpet – Phthalimide - Phthalic Acid - Phthalamic Acid
	Phthalimide Malt Sprouts, Dried Brewers Grain and Brewer's Yeast (quantitation): R: 0.9998 Calibration curve: y = 0.963 x + 0.0267 number of data points = 8 Phthalimide Beer (quantitation): R: 0.9999 Calibration curve: y = 0.956 x + 0.0305 number of data points = 8 Phthalic Acid (quantitation): R: 0.9991 Calibration curve: y = 0.301 x + 0.00394 number of data points = 8 Phthalamic Acid grain (quantitation): R: 0.9984 Calibration curve: y = 838134x + 2364 number of data points = 8 Phthalamic Acid brewing (Malt) (quantitation): R: 0.9988 Calibration curve: y = 363590 x + 643.74 number of data points = 8 Phthalamic Acid Malt Sprouts (quantitation): R: 0.9993 Calibration curve: y = 1.57e+005 x + 1.57e+003 number of data points = 8 Phthalamic Acid Dried Brewers Grain (quantitation): R: 0.9975 Calibration curve: y = 116948 x – 62.41 number of data points = 8 Phthalamic Acid Dried Brewers Grain (quantitation): R: 0.9992 Calibration curve: y = 4.08e+005 x + 8.22e+003 number of data points = 8 Phthalamic Acid Dried Beer(quantitation): R: 0.9990 Calibration curve: y = 3.82e+005 x + 8.46e+003 number of data points = 8
Calibration range	~~Folpet~~ ~~0.003 to 0.30 mg reference item/L~~ ~~Phthalimide~~ ~~0.003 to 0.75 mg reference item/L~~ Folpet and phthalimide Folpet and phthalimide in barley grain, brewing malt, malt sprouts, dried brewers grain and brewer's yeast 0.75 ng/mL to 75 ng/mL, corresponding to 0.003 mg/kg to 0.30 mg/kg Folpet and phthalimide in beer 3.0 ng/mL to 75 ng/mL, corresponding to 0.003 mg/kg to 0.075 mg/kg. Phthalic Acid ~~0.015 to 1.5 mg reference item/L~~ 3.75 ng/mL to 375 ng/mL, corresponding to 0.015 mg/kg to 1.5 mg/kg

	Folpet – Phthalimide - Phthalic Acid - Phthalamic Acid
	Phthalamic Acid ~~0.015 to 1.5 mg reference item/L~~ 1.875 ng/mL to 187.5 ng/mL for malt sprouts, corresponding to 0.015 mg/kg to 1.5 mg/kg 3.75 ng/mL to 375 ng/mL for barley grain, brewing malt, dried brewers grain, brewer's yeast and beer, corresponding to 0.015 mg/kg to 1.5 mg/kg
Assessment of matrix effects is presented	Yes
Limit of determination/quantification	For folpet and phthalimide in all matrices: LOQ = 0.01 mg/kg LOD = 0.003 mg/kg For phthalic acid and phthalamic acid in all matrices: LOQ = 0.05 mg/kg LOD = 0.015 mg/kg

Conclusion
With regard to selectivity, accuracy and precision, the analytical methods were applied successfully for set folpet, phtalamide, phatalic acid and phtalamic acid when analysing the samples of the study (brewing malt, malt sprouts, dried brewers grain, brewer's yeast and beer).

For folpet and phtalamide, the LOQ was ser at 0.01 mg/kg and the LOD at 0.003 mg/kg for all matrices, while for phtalic and phtalamic acids, the LOQ was set at 0.05 mg/kg and the LOD at 0.15 mg/kg.

The methods were successfully validated for the determination of folpet, phthalimide, phthalic acid and phthalamic acid according to the guidance documents SANTE/2020/12830, rev. 1 for risk assessment and/or monitoring and ENV/JM/MONO(2007)17. The method is also compliant with all the requirements of SANTE/2020/12830, rev. 2.

A 2.2.1.2.1 Analytical method 3

Comments of zRMS:	The method was evaluated by zRMS-Greece in RR of SAP50SCF (December 2023). zRMS-Greece comments: *The LC-MS/MS analytical method has been fully validated in dry commodities (see KCP 5.1.2/02) for the determination of residues of folpet and metabolites and was found acceptable.* zRMS-PL comments: The results achieved during the method validation have shown that the method for determination and confirmation of both analyte is fit for purpose as its performance is in accordance with requirements set on SANTE/2020/12830, Rev.1.

Reference:	KCP 5.1.2/10
Report	Validation of the Analytical Method for the Determination of Folpet and Metabolites Residues in Wheat, Gordo, J., 2022, Report No. VAL22/21
Guideline(s):	Guidelines for the generation of data concerning residues as provided in Annex II, part A, Section 6 and Annex III, Part A, Section 8 of Directive 91/414/EEC concerning the placing of plant protection products on the market. SANTE/2020/12830, Rev.1: Guidance Document on Pesticide Analytical Methods for Risk Assessment. and Post-approval Control and Monitoring Purposes, 24/02/2021; SANTE/12682/2019, Guidance document on analytical quality control and method validation procedures for pesticide residues analysis in food and feed, 01/01/2020. OECD Series on Testing and Assessment, Number 9.

Deviations:	No impact on the study
GLP:	Yes
Acceptability:	Fit for purpose

Materials and methods

For the determination of folpet and phthalimide residues in wheat grain, samples were extracted using ethyl acetate. The analyses were carried out by liquid chromatography coupled to mass spectrometry.

Extraction

Folpet and Phthalimide

5 g of homogeneous sample were weighed into a 50 mL polypropylene centrifuge tube and 15 mL of Milli-Q acidified water (1% formic acid) was added (fortification solution added here for spike tests). 10 mL of extraction solvent, ethyl acetate, was added and shaken manually for ≈ 1 minute. After this, 10 g of sodium sulfate anhydrous was added and shaken vigorously for some seconds, followed by other shaking step during ≈ 11 minutes on a mechanical shaker (Multi Reax). The obtained extract was subjected to dSPE cleanup using a mixture of 50 mg PSA + 150 mg Na2SO4 and shaken. The mixture was centrifuged for ≈ 5 minutes at ≈ 3000 rpm. The supernatant was then filtered through appropriate filters (PTFE, 0.20 µm). The supernatant (2 mL) was evaporated to dryness under a gentle stream of nitrogen, and reconstituted in 0.2 mL methanol, followed by a shaking step during ≈ 2 minutes on a mechanical shaker. Then, 0.8 mL of acidified water was added followed by another shaking step during ≈ 5 minutes on a mechanical shaker. An aliquot was transferred into a vial together with the same volume of mobile phase (first line LC gradient) for analysis.

Phthalic acid

5 g of homogeneous sample were weighed into a 50 mL polypropylene centrifuge tube and 4.5 mL of Milli-Q water was added (fortification solution added here for spike tests). 5 mL of extraction solvent, acidified methanol (1% formic acid), was added. Internal standard was added followed by a shaking step during ≈ 11 minutes on a mechanical shaker (Multi Reax). The mixture was centrifuged for ≈ 5 minutes at ≈ 4000 rpm. The supernatant was removed to a 50 mL polypropylene centrifuge tube. 5 mL of extraction solvent, acidified methanol (1% formic acid), was added to the remaining sample followed by a shaking step during ≈ 11 minutes on a mechanical shaker (Multi Reax). The mixture was centrifuged for ≈ 5 minutes at ≈ 4000 rpm. The supernatant was removed into the 50 mL polypropylene centrifuge tube with has collected the first extracted portion. Combined extracts were shaken manually. One part of the extract was transferred into a vial with three parts of volume of mobile phase (first line LC gradient) for analysis.

LC-QTRAP-conditions for folpet and phthalimide

LC-QTRAP System:	SCIEX Exion LC		
Column:	ACQUITY UPLC HSS T3 1.8 µm from Waters, 2.1 x 100 mm		
Oven temperature:	40 ºC		
Mobile Phase:	A: H2O:MeOH:1 M ammonium formate:formic acid (940:50:9:1, v/v) B: H2O:MeOH:1 M ammonium formate:formic acid (900:90:9:1, v/v)		
Gradient:	Time [min]	% A	% B
	0	95	5
	9	5	95
	13	95	5
Flow Rate:	0.4 mL / min		
Injection Volume:	10 µL		
Autosampler temperature:	15 ºC		
Integration Software:	SCIEX OS-MQ 3.1		

Mass spectrometric conditions

Folpet

Electrospray polarity: positive
Declustering Potential (DP): 50 V

MRM1 collision energy (259.9 > 129.9): 30 eV
MRM2 collision energy (259.9 > 102.0): 60 eV
MRM3 collision energy (261.9 > 129.9): 30 eV
Dwell time: 0.5 s
Typical Retention time: 9.1 min (with tolerance of ± 0.1 min)
Typical MRM Transition Ratio (MRM1/MRM2): 3.0 (with tolerance of ± 30 %)
Typical MRM Transition Ratio (MRM1/MRM3): 1.6 (with tolerance of ± 30 %)

Phthalimide
Electrospray polarity: negative
Declustering Potential: -50 V
MRM1 collision energy (146.0 > 42.0): -52 eV
Dwell time: 0.5 s
Typical Retention time: 4.5 min (with tolerance of ± 0.1 min)

LC-QTRAP-conditions for phthalic acid

LC-QTRAP System:	SCIEX Exion LC		
Column:	ACQUITY UPLC HSS T3 1.8 µm from Waters, 2.1 x 100 mm		
Oven temperature:	40 ºC		
Mobile Phase:	C: 0.1% formic acid in H2O D: 0.1% formic acid in meOH		
Gradient:	Time [min]	% C	% D
	0.00	70	30
	5.00	0	100
	5.50	70	30
	7.00	70	30
Flow Rate:	0.4 mL / min		
Injection Volume:	5 µL		
Autosampler temperature:	15 ºC		
Integration Software:	SCIEX OS-MQ 3.1		

Mass spectrometric conditions
Phthalic acid
Electrospray polarity: negative
Declustering Potential (DP): -5 V
MRM1 collision energy (164.9 > 121.0): -14 eV
MRM2 collision energy (164.9 > 77.0): -20 eV
Dwell time: 0.5 s
Typical Retention time: 1.6 min (with tolerance of ± 0.1 min)
Typical MRM Transition Ratio (MRM1/MRM2): 1.3 (with tolerance of ± 30 %)

Phthalic acid- d4
Electrospray polarity: negative
Declustering Potential (DP): -5 V
MRM1 collision energy (168.9 > 81.0): -22 eV

Results and discussions

Matrix effects
Matrix effects were studied and no significant matrix effects in LC-QTRAP were observed (< |20 %|) for both folpet and phthalimide. To quantify the spiked samples, matrix-matched standard solutions were used. Matrix effects wasn´t study for phthalic acid since the analysis were performed with internal standard (compensate for matrix effects).

Table A 14: Recovery results from method validation of folpet and phtalamide using the analytical method

Matrix	Analyte	Fortification level (mg/kg) (*n* = 5)*	Mean recovery (%)	RSD (%)	Comments
Wheat (grain)	Folpet	0.01	74.5	20.4	
Wheat (grain)	Folpet	0.1	75.9	8.8	
Wheat (grain)	Phthalimide	0.01	82.7	16.5	
Wheat (grain)	Phthalimide	0.1	91.8	18.1	

*For 0.10 mg/kg spike level for folpet, a fortified assay (EF9/94/VAL22/21/22) was excluded as it was considered an outlier.

Table A 15: Characteristics for the analytical method used for validation of folpet residues

	Folpet
Specificity	LC-QTRAP blank value < 30 % LOQ
Calibration (type, number of data points)	Correlation of peak area of different standard solutions with their corresponding concentrations, using a linear regression: Folpet: R: 0.99380 Calibration curve: y = 2.21008x + 642.78720 number of data points = 7 Phthalimide: R: 0.99912 Calibration curve: y = 5.33986 x + 430.98319 number of data points = 7
Calibration range	Folpet 0.0015 ng/µL to 0.0375 ng/µL, corresponding to 0.003 - 0.075 mg/kg Phthalimide 0.0015 ng/µL to 0.0375 ng/µL, corresponding to 0.003 - 0.075 mg/kg (MRM transition 146.0>42.0) 0.0015 ng/µL to 0.050 ng/µL, corresponding to 0.003 - 0.1 mg/kg (MRM transition 146.0>42.0)
Assessment of matrix effects is presented	Yes
Limit of determination/quantification	LOQ = 0.01 mg/kg LOD = 0.003 mg/kg

Conclusion
The method is successfully validated for the determination of folpet and phthalimide with LoQ of 0.01 mg/kg according to the guidance documents SANTE/2020/12830, rev. 1 for risk assessment. The method is also compliant with all the requirements of SANTE/2020/12830, rev. 2.

A 2.2.2 Methods for post-authorization control and monitoring purposes (KCP 5.2)

A 2.2.2.1 Description of analytical methods for the determination of residues in plant matrices (KCP 5.2)

A 2.2.2.1.1 Analytical method 1

Comments of zRMS:	The method was evaluated by zRMS-Greece in RR of SAP2101F (January 2024). zRMS-Greece comments:

	The study is considered acceptable.
Reference:	KCP 5.2/01
Report	Validation of the Analytical Method for the Determination of Folpet and Phthalimide in Grapes, Wine, Tomato, Cereal Grain and Sunflower Seeds, Perny, A., 2015, Report no. R B4225
Guideline(s):	SANCO/825/00 rev. 8.1 SANCO/3029/99 rev. 4
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Reference:	KCP 5.2/02
Report	Validation of the Analytical Method for the Determination of Folpet and Phthalimide in Grapes, Wine, Tomato, Cereal Grain and Sunflower Seeds – Amendment No. 1, Perny, A., 2015, Report no. R B4225
Guideline(s):	SANCO/825/00 rev. 8.1 SANCO/3029/99 rev. 4
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

Test item

Grapes, Tomato, Cereal grain, Sunflower seed

The method was also validated for wine, this was however not evaluated as it is not necessary for a monitoring method

Analyte

Folpet, Phthalimide

Principle of method

Homogenised plant material (approximately 10 g) is extracted with ethyl acetate and o-phosphoric acid in the presence of magnesium sulphate and sodium chloride. For sunflower an additional clean-up on a silica SPE cartridge is required. Folpet and phthalimide are determined concurrently by liquid-chromatography with MS/MS detector.

HPLC Conditions

- *Quantification:* column: BEH C18 (2.1 x 50 mm; particle size 1.7 µm)
  mobile phases: water/methanol; gradient mode
- *Confirmation:* column: ZORBAX SB-C3 (3 x 150 mm; particle size 5 µm)
  mobile phases: water/methanol; gradient mode

MS/MS Conditions

Quantification & Confirmation: m/z 146 → 41.9 (both analytes)

Results and discussions

Specificity/Interference

Due to the use of a highly specific detection system (MS/MS), the lack of significant (> 30 % of LOQ for the quantifier ion) interfering signals in the chromatograms and the identical retention times of the analyte in standard solutions and in extracts from samples, the procedure can be regarded to be highly specific for folpet and phthalimide.

Linearity
The linearity of the method was studied with matrix matched standards (n=7) between 3 ng/mL and 120 ng/mL (corresponding to 0.003 to 0.12 in mg/kg) of folpet and phthalimide in grapes, tomato, cereal grain and sunflower seeds. The linear correlation coefficients were > 0.990, showing a good linearity.
Plots of the graphs and parameter of the equations are available.

Accuracy
For quantification the samples are fortified at 0.01 and 0.1 mg/kg for both analytes. For confirmation only a fortification at 0.01 mg/kg is presented (fortification at 0.01 and 0.1 mg/kg is presented in the ILV). 5 recoveries per concentration are determined.
Mean recovery is between 70 and 120 %.

Repeatability
The relative standard deviation (RSD) over all fortification levels is within guideline requirements of RSD (< 20% for 0.1 mg/kg and < 30% for 0.01 mg/kg).

Matrix effects
Matrix effects on the detection of folpet and phthalimide in extracts of grapes and tomato were found to be significant (> ± 20%). Matrix matched standards were used for quantification for all matrices, by default.

Extraction efficiency
The extraction efficiency of the method in grapes and tomatoes has been investigated in a separate study (Ertus, 2016). The extraction efficiency in other crop groups could not be investigated due to lack of crop samples with incurred residues.
The conclusion of this study is as follows:
Extractions of the identical field samples of grapes and tomato with incurred residues using different solvent systems yielded comparable residue levels. It is therefore concluded that the efficiency of one extraction with ethyl acetate plus concentrated o-phosphoric acid is proven for residues of folpet and phthalimide in grapes and tomato fruit (detailed results are given in Volume 3 CA B-7, chapter 7.7.1).

LOQ: 0.01 mg/kg for folpet and phthalimide in all matrices.

Table A 14: Recovery results from method validation of folpet and phthalimide using the analytical method

Matrix	Analyte	Fortification level (mg/kg) (*n* = 5)	Mean recovery (%)	RSD (%)	Comments
Grapes	Folpet	0.01	90.3	10.2	*Quantification* BEH C18 column *m/z* 146.0 → 41.9
		0.10	103.6	6.0	
		0.01	104.7	5.4	*Confirmation* ZORBAX SB-C3 column *m/z* 146.0 → 41.9
	Phthalimide	0.01	90.5	1.6	*Quantification* BEH C18 column *m/z* 146.0 → 41.9
		0.10	96.8	6.9	
		0.01	95.6	3.5	*Confirmation* ZORBAX SB-C3 column

Matrix	Analyte	Fortification level (mg/kg) (*n* = 5)	Mean recovery (%)	RSD (%)	Comments
					m/z 146.0 → 41.9
Tomato	Folpet	0.01	107.8	2.0	*Quantification* BEH C18 column *m/z* 146.0 → 41.9
		0.10	106.9	4.4	
		0.01	93.6	14.5	*Confirmation* ZORBAX SB-C3 column *m/z* 146.0 → 41.9
	Phthalimide	0.01	102.9	5.8	*Quantification* BEH C18 column *m/z* 146.0 → 41.9
		0.10	97.2	2.8	
		0.01	105.1	4.0	*Confirmation* ZORBAX SB-C3 column *m/z* 146.0 → 41.9

Table A 154b: Recovery results from method validation of folpet and phthalimide using the analytical method

Summary of recoveries - folpet

Analyte	Matrix	Fortification level (mg/kg)	Mean recovery Percentage (%)	Standard deviation (SD) (%)	Relative standard deviation (RSD) (%)	Number of fortified samples (n)
Folpet	Grapes	0.01	90.3%	9.2%	10.2%	5
		0.10	103.6%	6.3%	6.0%	5
		All levels	96.9%	10.2%	10.5%	10
Folpet	Wine	0.01	94.1%	4.8%	5.1%	5
		0.10	92.6%	8.6%	9.3%	5
		All levels	93.4%	6.6%	7.1%	10
Folpet	Tomato	0.01	107.8%	2.2%	2.0%	5
		0.10	106.9%	4.7%	4.4%	5
		All levels	107.3%	3.5%	3.2%	10
Folpet	Cereal grain	0.01	76.9%	3.8%	5.0%	5
		0.10	72.9%	2.4%	3.4%	5
		All levels	74.9%	3.7%	4.9%	10
Folpet	Sunflower seeds	0.01	74.9%	4.3%	5.7%	5
		0.10	81.9%	5.9%	7.2%	5
		All levels	78.4%	6.1%	7.8%	10

Table A 164c: Recovery results from method validation of folpet and phthalimide using the analytical method

Summary of recoveries - phthalimide

Analyte	Matrix	Fortification level (mg/kg)	Mean recovery Percentage (%)	Standard deviation (SD) (%)	Relative standard deviation (RSD) (%)	Number of fortified samples (n)
Phthalimide	Grapes	0.01	90.5%	1.4%	1.6%	5
		0.10	96.8%	6.7%	6.9%	5
		All levels	93.7%	5.6%	6.0%	10
Phthalimide	Wine	0.01	110.2%	3.2%	2.9%	5
		0.10	109.4%	2.9%	2.7%	5
		All levels	109.8%	2.9%	2.6%	10
Phthalimide	Tomato	0.01	102.9%	6.0%	5.8%	5
		0.10	97.2%	2.7%	2.8%	5
		All levels	100.1%	5.3%	5.3%	10
Phthalimide	Cereal grain	0.01	98.9%	3.8%	3.8%	5
		0.10	101.7%	3.7%	3.6%	5
		All levels	100.3%	3.8%	3.8%	10
Phthalimide	Sunflower seeds	0.01	72.6%	1.3%	1.8%	5
		0.10	77.4%	1.5%	1.9%	5
		All levels	75.0%	2.8%	3.8%	10

Table A 17: Characteristics for the analytical method used for validation of folpet residues in plant matrices

	Folpet	Phthalimide
Specificity	LC-MS/MS Primary method: *m/z* 146.0 → 41.9 (Column: BEH C18) Confirmatory method: *m/z* 146.0 → 41.9 (Column: ZORBAX SB-C3) blank value < 30 % LOQ	LC-MS/MS Primary method: *m/z* 146.0 → 41.9 (Column: BEH C18) Confirmatory method: *m/z* 146.0 → 41.9 (Column: ZORBAX SB-C3) blank value < 30 % LOQ
Calibration (type, number of data points)	Grapes: Primary method: C=2.2834E-03×S + 1.32 (r=0.99760) Confirmatory method: C=2.7252E-03×S + 0.99 (r=0.99907) Tomato: Primary method: C=3.1875E-03×S + 1.15 (r=0.99914) Confirmatory method: C=2.6706E-03×S + 0.97 (r=0.99955) Folpet Grapes C (Concentration) = 2.2834E-03 x S (Peak area) + 1.32 r = 0.99760 Phthalimide Grapes C (Concentration) = 5.3098E-04 x S (Peak area) – 4.43 r = 0.99996 Folpet – Wine C (Concentration) = 3.5615E-03 x S (Peak area) + 0.63 r = 0.99993 Phthalimide – Wine C (Concentration) = 5.4026E-04 x S (Peak area) - 1.82 r = 0.99986 Folpet – Tomato C (Concentration) = 3.1875E-03 x S (Peak area) + 1.15 r = 0.99914 Phthalimide – Tomato C (Concentration) = 5.3363E-04 x S (Peak area) + 0.15 r = 0.99994 Folpet - Cereal grain C (Concentration) = 3.6044E-03 x S (Peak area) + 0.40 r = 0.99976 Phthalimide - Cereal grain C (Concentration) = 5.9062E-04 x S (Peak area) + 0.07 r = 0.99998 Folpet - Sunflower seeds C (Concentration) = 1.6512E-02 x S (Peak area) + 1.08 r = 0.99949 Phthalimide - Sunflower seeds C (Concentration) = 1.6454E-03 x S (Peak area) - 1.74 r = 0.99926 8 data points	Grapes: Primary method: C=5.3098E-04×S – 4.43 (r=0.99996) Confirmatory method: C=5.5002E-04×S – 1.94 (r=0.99988) Tomato: Primary method: C=5.3363E-04×S + 0.15 (r=0.99994) Confirmatory method: C=5.1609E-04×S + 0.59 (r=0.99979) 8 data points

	Folpet	Phthalimide
Calibration range	Accepted calibration range in concentration units: 3 – 121 ng/mL Corresponding calibration range in mass ratio units for the sample: 0.003 – 0.12 mg/kg	Accepted calibration range in concentration units: 3 – 120 ng/mL Corresponding calibration range in mass ratio units for the sample: 0.003 – 0.12 mg/kg
Assessment of matrix effects is presented	yes	yes
Limit of determination/quantification	LOQ = 0.01 mg/kg LOD = 0.003 mg/kg	LOQ = 0.01 mg/kg LOD = 0.003 mg/kg
Stability	The stability of extracts during storage at 4°C was investigated. The results indicate a good stability for up to 4 days for grapes, tomato and cereal grain, 7 days for wine and 3 days for sunflower seeds. Stability results for standard solutions in methanol/H2O 90:10 + 0.1% formic acid The stability of standard solutions during storage at 4°C was investigated. The results indicate a good stability for up to 4 days in methanol/H2O 90:10 + 0.1% formic acid. Stability results for matrix matched standard solutions in sunflower seeds. The stability of matrix matched standard solutions during storage at 4°C was investigated. The results indicate a good stability for up to 3 days for sunflower seeds.	

Conclusion
The residue method for folpet and phthalimide in grapes, tomato, cereal grain, and sunflower seeds was successfully validated. Limit of quantification is 0.01 mg/kg for both folpet and phthalimide. All validation parameters are within the limit values defined by the corresponding European guidance document SANCO/825/00 rev 8.1. All parameters are also according to the new guidance SANTE/2020/12830 rev. 2. The analyses were carried out by LC-MS/MS, using two different columns for quantification and confirmation.
~~Due to low recoveries obtained in the independent lab validation, the method for the analysis of both analytes with both primary and confirmatory method in cereal grain and sunflower seed could not be successfully validated according to the guidance document SANCO/825/00 rev 8.1 with a LOQ of 0.01 mg/kg. Therefore, the method is not appropriate for the determination of folpet and phthalimide in cereal grain and sunflower seed.~~

A 2.2.2.1.1.1 Independent laboratory validation

Comments of zRMS:	The method was evaluated by zRMS-Greece in RR of SAP2101F (January 2024). zRMS-Greece comments: *The study is considered acceptable as ILV of the method R B4225 for grapes and tomato.*

Reference:	KCP 5.2/03
Report	Independent laboratory validation of the analytical method for the determination of folpet and phthalimide in crop matrices by LC-MS/MS, Meseguer, 2016, Report no: S14-05779
Guideline(s):	SANCO/825/00 rev. 8.1 SANCO/3029/99 rev. 4 OECD ENV/JM/MONO(2007)17
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

Test item

Grapes, Tomato, Cereal grain, Sunflower seed

The method was also validated for wine, this was however not evaluated as it is not necessary for a monitoring method

Analyte

Folpet, Phthalimide

HPLC Conditions

- *Quantification:* column: BEH C18 (2.1 x 50 mm; particle size 1.7 µm)
  mobile phases: water/methanol; gradient mode
- *Confirmation:* column: ZORBAX SB-C3 (3 x 150 mm; particle size 5 µm)
  mobile phases: water/methanol; gradient mode

MS/MS Conditions

Quantification & Confirmation: m/z 146 → 41.9 (both analytes)

Results and discussions

An independent laboratory validation was conducted for all 4 matrices. Analysis of samples was performed and detected according to the primary method.

The method was found to be valid according to the guidance documents SANCO/825/00 rev 8.1 for the determination of folpet and phthalimide with both primary and confirmatory method in grapes and tomato with a LOQ of 0.01 mg/kg.

Due to low recoveries obtained, the method for the analysis of both analytes with both primary and confirmatory method in cereal grain and sunflower seed could not be successfully validated according to the guidance document SANCO/825/00 rev 8.1 with a LOQ of 0.01 mg/kg.

Table A 18: Recovery results from independent laboratory validation of folpet and phthalimide using the analytical method

Matrix	Analyte	Fortification level (mg/kg) (*n* = 5)	Mean recovery (%)	RSD (%)	Comments
Grapes	Folpet	0.01	103	7	*Quantification:* BEH C18 column *m/z* 146.0 → 41.9
		0.10	107	5	
		0.01	106	11	*Confirmation:* ZORBAX SB-C3 column; *m/z* 146.0 → 41.9
		0.10	104	5	
	Phthalimide	0.01	93	8	*Quantification:* BEH C18 column *m/z* 146.0 → 41.9
		0.10	93	6	
		0.01	91	8	*Confirmation:* ZORBAX SB-C3 column; *m/z* 146.0 → 41.9
		0.10	98	8	
Tomato	Folpet	0.01	107	5	*Quantification:* BEH C18 column *m/z* 146.0 → 41.9
		0.10	108	4	
		0.01	90	8	*Confirmation:* ZORBAX SB-C3 column; *m/z* 146.0 → 41.9
		0.10	93	8	
	Phthalimide	0.01	74	6	*Quantification:* BEH C18 column *m/z* 146.0 → 41.9
		0.10	87	1	
		0.01	77	7	*Confirmation:* ZORBAX SB-C3 column;

Matrix	Analyte	Fortification level (mg/kg) (*n* = 5)	Mean recovery (%)	RSD (%)	Comments
		0.10	95	4	*m/z* 146.0 → 41.9

Table A 19: Characteristics for the analytical method used for independent laboratory validation of folpet residues in plant matrices

	Folpet	Phthalimide
Specificity	LC-MS/MS Primary method: *m/z* 146.0 → 41.9 (Column: BEH C18) Confirmatory method: *m/z* 146.0 → 41.9 (Column: ZORBAX SB-C3) blank value < 30 % LOQ	LC-MS/MS Primary method: *m/z* 146.0 → 41.9 (Column: BEH C18) Confirmatory method: *m/z* 146.0 → 41.9 (Column: ZORBAX SB-C3) blank value < 30 % LOQ
Calibration (type, number of data points)	Grapes: Primary method: y=105x-222 (r^2=0.9946) 8 data points Confirmatory method: y=20976x-27701 (r^2=0.9958) 8 data points Tomato: Primary method: y=161x-69 (r^2=0.9920) 7 data points Confirmatory method: y=19380x-18631 (r^2=0.9992) 8 data points	Grapes: Primary method: y=245x-279 (r^2=0.9944) 7 data points Confirmatory method: y=40519x+79606 (r^2=0.9994) 8 data points Tomato: Primary method: y=700x+1050 (r^2=0.9938) 8 data points Confirmatory method: y=56202x+199032 (r^2=0.9964) 7 data points
Calibration range	Accepted calibration range in concentration units: 3 – 120 ng/mL Corresponding calibration range in mass ratio units for the sample: 0.003 – 0.12 mg/kg	Accepted calibration range in concentration units: 3-120 ng/mL Corresponding calibration range in mass ratio units for the sample: 0.003-0.12 mg/kg
Assessment of matrix effects is presented	yes	yes
Limit of determination / quantification	Grapes and tomato: LOQ = 0.01 mg/kg LOD = 0.003 mg/kg	Grapes and tomato: LOQ = 0.01 mg/kg LOD = 0.003 mg/kg

Conclusion
The method was found to be valid according to the guidance documents SANCO/825/00 rev 8.1 for the determination of folpet and phthalimide with both primary and confirmatory method in grapes and tomato with a LOQ of 0.01 mg/kg. All parameters are also according to the new guidance SANTE/2020/12830 rev. 2.
Once the method from report no. R B4225 could not be validated by an independent laboratory for the determination of folpet and phthalimide in cereal grain and sunflower seed, the multi-residue method DFG S19 was additionally validated for the analysis of folpet and phthalimide in these crop matrices.

A 2.2.2.1.2 Analytical method 2

Comments of zRMS:	The method was evaluated by zRMS-Greece in RR of SAP2101F (January 2024). zRMS-Greece comments: *The study is considered acceptable as ILV of the method R B4225 for barley grain and*

	sunflower seed.

Reference:	KCP 5.2/04
Report	Validation of the multi-residue method DFG-S19 for the determination of folpet and phthalimide in cereal grain and sunflower seeds. Wiesner F., Breyer N., 2016, Report no: S16-00559 (BEL-1601V)
Guideline(s):	SANCO/825/00 rev. 8.1 SANCO/3029/99 rev. 4 OPPTS 860.1340 OECD ENV/JM/MONO(2007)17
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Reference:	KCP 5.2/05
Report	Validation of the multi-residue method DFG-S19 for the determination of folpet and phthalimide in cereal grain and sunflower seeds – Amendment No.1. Wiesner F., Breyer N., 2016, Report no: S16-00559 (BEL-1601V)
Guideline(s):	SANCO/825/00 rev. 8.1 SANCO/3029/99 rev. 4
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods
Test item
Cereal grain, Sunflower seed

Analyte
Folpet, Phthalimide

Principle of method
Samples of cereal grain were extracted with acetone according to multi-residue method DFG S19 module E2. Before the addition of acetone, acidified warm water was added in an amount that takes full account of the natural water content of the specimen - so that the acetone/water ratio during extraction is 2/1 (v/v). For liquid-liquid partition, ethyl acetate/cyclohexane (1/1, v/v) and sodium chloride were added, and after repeated mixing excess water was separated. An aliquot of the organic phase was evaporated to a dry residue.
Samples of sunflower seeds were extracted with acetone/acetonitrile in a glass jar containing Calflo E and Celite according to multi-residue method DFG S19 module E7. The suspension was mixed well and filtered with suction through a Buchner porcelain funnel equipped with a round paper filter. Afterwards, the filtrate was filtered through a dry fluted filter equipped with 0.5 g Calflo E into a graduated measuring cylinder. After addition of iso-octane, the extract was reduced using rotary-evaporation.
The residues obtained from extraction module E7 for sunflower seeds and extraction module E2 for cereal grain were cleaned up by gel permeation chromatography on Bio Beads S-X3 polystyrene gel using a mixture of ethyl acetate/cyclohexane (1/1, v/v) as eluant. The fraction containing phthalimide and folpet residues was concentrated to dryness. After reconstitution in acetonitrile/1% acetic acid (3/7, v/v), the final extracts of cereal grain and sunflower seeds were analysed for folpet and phthalimide by liquid chromatography using tandem mass spectrometric detection (LC-MS/MS).

HPLC Conditions

Column: Develosil RP Aqueous-3 140A (150 x 3.0 mm; particle size 3.0 µm)
Mobile phases: water / methanol (both with 0.5% formic acid); gradient mode

MS/MS Conditions

Quantification: *m/z* 298 → 260 (Folpet)

m/z 148 → 130 (Phthalimide)

Confirmation: *m/z* 296 → 130 (Folpet)

m/z 148 → 102 (Phthalimide)

Results and discussions

Specificity/Interference

Due to the use of a highly specific detection system (MS/MS), the lack of significant (> 30 % of LOQ for the quantifier ion) interfering signals in the chromatograms and the identical retention times of the analyte in standard solutions and in extracts from samples, the procedure can be regarded to be highly specific for Folpet and Phthalimide.

Linearity

The linearity of the method was studied with matrix matched standards between 1.0 ng/mL and 200 ng/mL (corresponding to 0.0021 to 0.43 in mg/kg for grain and 0.0025 to 0.33 mg/kg for sunflower seeds) of folpet and phthalimide in barley grain and sunflower seeds. The linear correlation coefficients were > 0.990, showing a good linearity.
Plots of the graphs and parameter of the equations are available.

Accuracy

For quantification the samples are fortified at 0.01 and 0.1 mg/kg for both analytes. 5 recoveries per concentration are determined.
Mean Recovery is between 60 and 120 %.

Repeatability

The relative standard deviation (RSD) over all fortification levels is within guideline requirements of RSD (< 20% for 0.1 mg/kg and < 30% for 0.01 mg/kg).

Matrix effects

Matrix effects on the detection of folpet and phthalimide in extracts of barley grain were found to be significant and therefore matrix-matched standards were used for quantification. Matrix effects on the detection of folpet in extracts of sunflower seeds were found to be significant, therefore matrix-matched standards were used for quantification. Matrix effects on the detection of phthalimide in extracts of sunflower seeds were found to be insignificant, therefore solvent standard solutions were used for quantification.

LOQ: 0.01 mg/kg for folpet and phthalimide in dry and oily matrix.

Table A 20: Recovery results from method validation of folpet and phthalimide using the analytical method

Matrix	Analyte	Fortification level (mg/kg) (*n* = 5)	Mean recovery (%)	RSD (%)	Comments
Barley grain	Folpet	0.01	100	8.6	*Quantification* *m/z* 298 → 260
		0.10	89	2.6	
		0.01	101	9.1	*Confirmation* *m/z* 296 → 130
		0.10	91	1.8	

Matrix	Analyte	Fortification level (mg/kg) (*n* = 5)	Mean recovery (%)	RSD (%)	Comments
	Phthalimide	0.01	97	12	*Quantification* *m/z* 148 → 130
		0.10	98	5.1	
		0.01	95	11	*Confirmation* *m/z* 148 → 102
		0.10	95	4.2	
Sunflower seeds	Folpet	0.01	95	12	*Quantification* *m/z* 298 → 260
		0.10	81	11	
		0.01	95	11	*Confirmation* *m/z* 296 → 130
		0.10	80	11	
	Phthalimide	0.01	86	17	*Quantification* *m/z* 148 → 130
		0.10	106	2.7	
		0.01	89	19	*Confirmation* *m/z* 148 → 102
		0.10	110	2.4	

Table A 21: Characteristics for the analytical method used for validation of folpet residues in plant matrices

	Folpet	Phthalimide
Specificity	LC-MS/MS Primary method: *m/z* 298 → 260 Confirmatory method: *m/z* 296 → 130 blank value < 30 % LOQ	LC-MS/MS Primary method: *m/z* 148 → 130 Confirmatory method: *m/z* 148 → 102 blank value < 30 % LOQ
Calibration (type, number of data points)	Barley grain: Primary method: y=1870.7952x + 1485.1669 (r=0.9997) Confirmatory method: y=3099.4006x + 2092.5616 (r=0.9999) 6 data points Sunflower seeds: Primary method: y=3772.8481x + 2601.5185 (r=0.9998) Confirmatory method: y=6706.3128x + 5697.6074 (r=0.9998) 7 data points	Barley grain: Primary method: y=22397.6047x – 1266.5626 (r=0.9996) Confirmatory method: y=14229.7766x – 3666.1278 (r=0.9995) 6 data points Sunflower seeds: Primary method: y=24557.5986x – 24804.7222 (r=0.9999) Confirmatory method: y=15037.4493x – 12136.4874 (r=0.9999) 7 data points
Calibration range	Accepted calibration range in concentration units: 1.0 – 200 ng/mL Corresponding calibration range in mass ratio units for the sample: Barley grain: 0.0021 – 0.43 mg/kg Sunflower seeds: 0.0025 – 0.33 mg/kg	Accepted calibration range in concentration units: 1.0 – 200 ng/mL Corresponding calibration range in mass ratio units for the sample: Barley grain: 0.0021 – 0.43 mg/kg Sunflower seeds: 0.0025 – 0.33 mg/kg
Assessment of matrix effects is presented	yes	yes
Limit of determination/quantification	LOQ = 0.01 mg/kg LOD = 0.003 mg/kg	LOQ = 0.01 mg/kg LOD = 0.003 mg/kg

Conclusion

The multi-residue method DFG S19 was successfully validated by an independent laboratory for the analysis of folpet and phthalimide in/on sunflower seed and cereal grain at the tested LOQ of 0.01 mg/kg

according to the guidance document SANCO/825/00 rev. 8.1. Moreover, this method is also valid according to the new guidance SANTE/2020/12830 rev.2.

A 2.2.2.1.2.1 Independent laboratory validation

Comments of zRMS:	The method was evaluated by zRMS-Greece in RR of SAP50SCF (December 2023). zRMS-Greece comments: *The study is considered acceptable.* *However, it has to be mentioned that the RSD values in the case of determination of phthalimide in sunflower seeds is slightly above 20%.*

Reference:	KCP 5.2/06
Report	Independent Laboratory Validation of the analytical method for the determination of folpet and phthalimide in cereal grain and sunflower seeds. Hegmanns, C., 2016, Report no: S16-00716
Guideline(s):	SANCO/825/00 rev. 8.1 SANCO/3029/99 rev. 4
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods
Test item
Wheat grain, Sunflower seed

Analyte
Folpet, Phthalimide

Results and discussions
An independent laboratory validation was conducted for 2 matrices. Matrix effects on the detection of folpet and phthalimide in extracts of sunflower seeds and of folpet in extracts of wheat grain were found to be significant (≥ 20 %). Therefore, matrix-matched standards were used for quantification. Matrix effects on the detection of phthalimide in extracts of wheat grain were found to be insignificant (< 20 %). However, matrix-matched standards were used for quantification.
Analysis of samples was performed and detected according to the primary method differing slightly in calibration range but still in line with SANCO/825/00 rev 8.1.

Table A 22: Recovery results from independent laboratory validation of folpet and phthalimide using the analytical method

Matrix	Analyte	Fortification level (mg/kg) (*n* = 5)	Mean recovery (%)	RSD (%)	Comments
Sunflower seeds	Folpet	0.01	81	15	*Quantification* *m/z* 298 → 260
		0.1	77	11	
		0.01	81	14	*Confirmation* *m/z* 298 → 130
		0.1	78	11	
	Phthalimide	0.01	89	26	*Quantification* *m/z* 148 → 130
		0.1	101	16	
		0.01	82	27	*Confirmation*

Matrix	Analyte	Fortification level (mg/kg) (*n* = 5)	Mean recovery (%)	RSD (%)	Comments
		0.1	106	19	*m/z* 148 → 102
Wheat grain	Folpet	0.01	60	7	*Quantification* *m/z* 298 → 260
		0.1	71	7	
		0.01	62	11	*Confirmation* *m/z* 298 → 130
		0.1	70	7	
	Phthalimide	0.01	79	17	*Quantification* *m/z* 148 → 130
		0.1	93	6	
		0.01	79	6	*Confirmation* *m/z* 148 → 102
		0.1	91	9	

Table A 23: Characteristics for the analytical method used for independent laboratory validation of folpet residues in plant matrices

	Folpet	Phthalimide
Specificity	LC-MS/MS Primary method: *m/z* 298 → 260 Confirmatory method: *m/z* 298 → 130 blank value < 30 % LOQ	LC-MS/MS Primary method: *m/z* 148 → 130 Confirmatory method: *m/z* 148 → 102 blank value < 30 % LOQ
Calibration (type, number of data points)	Sunflower seeds: Primary method: y=1.61e+004x – 1.08e+003 (r=0.9999) Confirmatory method: y=2.33e+004x – 1.64e+003 (r=0.9998) 7 data points Wheat grain: Primary method: y=1.53e+004x + 2.05e+003 (r=0.9989) Confirmatory method: y=2.19e+004x – 7.59e+003 (r=0.9987) 6 data points	Sunflower seeds: Primary method: y=9.69e+004x + 2.1e+005 (r=0.9990) Confirmatory method: y=6.01e+004x + 1.38e+005 (r=0.9999) 7 data points Wheat grain: Primary method: y=8.33e+004x + 3.82e+004 (r=0.9987) Confirmatory method: y=5.34e+004x + 4.41e+004 (r=0.9988) 6 data points
Calibration range	Accepted calibration range in concentration units: 1.5 – 100 ng/mL Corresponding calibration range in mass ratio units for the sample: 0.003 – 0.2 mg/kg	Accepted calibration range in concentration units: 1.5 – 100 ng/mL Corresponding calibration range in mass ratio units for the sample: 0.003 – 0.2 mg/kg
Assessment of matrix effects is presented	yes	yes
Limit of determination/quantification	LOQ=0.01 mg/kg LOD=0.003 mg/kg	LOQ=0.01 mg/kg LOD=0.003 mg/kg

Conclusion
The method was successfully validated for all analytes and matrices at the tested LOQ of 0.01 mg/kg according to the guidance document SANCO/825/00 rev. 8.1. Furthermore, this method is also valid according to the new guidance SANTE/2020/12830 rev.2.

A 2.2.2.1.2.2 Extraction efficiency

Comments of zRMS:	Wheat grain samples with incurred residues of folpet and metabolites were extracted with both extraction conditions, the one applied during the ^{14}C-metabolism studies and the extraction conditions of the method validated under the scope of LabRP GLP studies (VAL22/21), in order to evaluate the extraction efficiency. The extraction efficiency was sufficiently proven since the difference between the two methods was lower than 30% for all analytes quantifiable. This is in accordance with requirements set on SANTE/2017/10632, Rev. 4, 23 February 2022. The cross validation is acceptable.

Reference:	KCP 5.2/16
Report	Cross validation of an internal extraction method from LabRP vs. an Extraction Method Applied in 14C-metabolism Studies for the Determination of Folpet and Metabolites in Wheat, Gordo, J., 2023, Report No. VAL25/21
Guideline(s):	OECD Series on Principles of GLP and Compliance Monitoring: Number 1, OECD Principles on Good Laboratory Practice (as revised in 1997) (ENV/MC/CHEM(98)17). Directive 2004/10/EC (codified version) from European Parliament and Council of 11 February 2004. Decreto-Lei nº 99/2000 of 30 May 2000 (Portuguese decree on OECD Principles of GLP).
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

The objective of the current study was to perform a cross validation between a method validated under Laboratório de Resíduos de Pesticidas (LabRP) GLP study VAL22/21 and the extraction conditions used in the ^{14}C-metabolism studies, for the determination of folpet, phthalimide and phthalic acid, in wheat (grain).

This evaluation was performed by extraction of incurred samples using both methods. The samples were generated during SGS study 21-00156 under the direction of Anne Sophie Beaulavon (wheat grain sample 322/VAL25/21/22 was used). The absence of folpet, phthalimide and phthalic acid in the untreated samples was checked prior to the quantification of spiked samples.

A method validation was performed in the scope of this study for the extraction conditions used in ^{14}C-metabolism studies and, samples were extracted in those conditions. These validations were performed according to SANTE/2020/12830 rev.1 "Guidance Document on Pesticide Analytical Methods for Risk Assessment and Post-approval Control and Monitoring Purposes, 24/02/2021".
Results were compared with the results obtained using the extraction methods validated under the LabRP GLP quantification study VAL22/21.

<u>Extraction ^{14}C-Metabolism method</u>

5 g of homogeneous sample were weighed into a 50 mL polypropylene centrifuge tube and fortification solution added here for spike tests. 10 mL of a solution of ethyl acetate:water:phosphoric acid (70:30:1.2 v/v/v) was added and shaken during ≈ 11 minutes on a mechanical shaker (Multi Reax). The obtained extract was centrifuged for ≈ 5 minutes at ≈ 4000 rpm. The supernatant was transferred to a 50 mL polypropylene centrifuge tube. To the sample, 5 mL a solution of acetonitrile:water:phosphoric acid (70:30:0.2 v/v/v) was added, shaken during ≈ 11 minutes on a mechanical shaker (Multi Reax) and centrifuged for ≈ 5 minutes at ≈ 4000 rpm. This second supernatant was added to the first supernatant and this mixture taken under nitrogen stream until the complete evaporation of the organic phase. After that the extract was transferred to a 10 mL measuring cylinder and the 10mL volume was made with a solution 95% water:5% methanol acidified with 0.1% formic acid.

An aliquot was transferred into a vial together with the same volume of mobile phase (first line LC gradient) for analysis.

Extraction VAL22/21 methods

Folpet and phthalimide determination:
5 g of homogeneous sample were weighed into a 50 mL polypropylene centrifuge tube and 15 mL of Milli-Q acidified water (1% formic acid) was added (fortification solution added here for spike tests). 10 mL of extraction solvent, ethyl acetate, was added and shaken manually for ≈ 1 minute. After this, 10 g of sodium sulphate anhydrous was added and shaken vigorously for some seconds, follow by other shaking step during ≈ 11 minutes on a mechanical shaker (Multi Reax). The obtained extract was subjected to dSPE clean-up using a mixture of 50 mg PSA + 150 mg Na2SO4 and shaken. The mixture was centrifuged for ≈ 5 minutes at ≈ 3000 rpm. The supernatant was then filtered through appropriate filters (PTFE, 0.20 µm). The supernatant (2 mL) was evaporated to dryness under a gentle stream of nitrogen, and reconstituted in 0.2 mL methanol, followed by a shaking step during ≈ 2 minutes on a mechanical shaker. Then, 0.8 mL of acidified water was added followed by another shaking step during ≈ 5 minutes on a mechanical shaker.
An aliquot was transferred into a vial together with the same volume of mobile phase (first line LC gradient) for analysis.

Phthalic acid determination:
5 g of homogeneous sample were weighed into a 50 mL polypropylene centrifuge tube and 4.5 mL of Milli-Q water was added (fortification solution added here for spike tests). 5 mL of extraction solvent, acidified methanol (1% formic acid), was added. Internal standard was added followed by a shaking step during ≈ 11 minutes on a mechanical shaker (Multi Reax). The mixture was centrifuged for ≈ 5 minutes at ≈ 4000 rpm. The supernatant was removed to a 50 mL polypropylene centrifuge tube. 5 mL of extraction solvent, acidified methanol (1% formic acid), was added to the remaining sample followed by a shaking step during ≈ 11 minutes on a mechanical shaker (Multi Reax). The mixture was centrifuged for ≈ 5 minutes at ≈ 4000 rpm. The supernatant was removed into the 50 mL polypropylene centrifuge tube with has collected the first extracted portion. Combined extracts were shaken manually.
One part of the extract was transferred into a vial with three parts of volume of mobile phase (first line LC gradient) for analysis

Results

Sample code	Analyte	Plot	**14C-metabolism method** [Mean value (mg/kg) +/- RSD (%)]	**VAL22/21 method** [Mean value (mg/kg) +/- RSD (%)]
322/VAL25/21/22	Folpet	Untreated	< LOQ	< LOQ
1839/VAL25/21		Treated	0.014 +/-7.2%	0.016 +/-7.4%
322/VAL25/21/22	Phthalamide	Untreated	< LOQ	< LOQ
1839/VAL25/21		Treated	0.014 +/-4%	0.016 +/- 6.3%
322/VAL25/21/22	Phthalic Acid	Untreated	< LOQ	< LOQ
1839/VAL25/21		Treated	0.35 +/-5.6%	0.34 +/- 3.4%

Conclusions
The extraction efficiency was sufficiently proven since the difference between the two methods was lower than 30% for all analytes quantifiable.

A 2.2.2.2 Description of analytical methods for the determination of residues in animal matrices (KCP 5.2)

A 2.2.2.2.1 Analytical method 1

Comments of zRMS:	The method was evaluated by zRMS-Greece in RR of SAP2101F (January 2024).

	zRMS-Greece comments: *The method is considered acceptable.*

Reference:	KCP 5.2/09
Report	Validation of the analytical method for the determination of phthalimide, expressed as folpet, in milk, eggs, meat, fat and liver/kidney, Schlewitz, P., 2015, report no: R B4281
Guideline(s):	SANCO/825/00 rev. 8.1 SANCO/3029/99 rev. 4
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods
Test item
Muscle, liver, fat, egg, milk

Analyte
Phthalimide

Principle of method
Homogenised samples (approximately 10 g) are extracted with acetone and o-phosphoric acid in the presence of magnesium sulphate and sodium chloride. For milk an additional clean-up on a silica SPE cartridge is required. Phthalimide is determined by liquid-chromatography with MS/MS detector.

HPLC Conditions

Quantification: Column: BEH C18 (2.1 x 50 mm; particle size 1.7 µm)
Mobile phases: water / methanol; gradient mode

Confirmation: Column: ZORBAX SB-C3 (3 x 150 mm; particle size 5.0 µm)
Mobile phases: water / methanol; gradient mode

MS/MS Conditions

Quantification & Confirmation: m/z 146 → 41.9
Results and discussions

Specificity/Interference
Due to the lack of significant (> 30 % of LOQ for the quantifier ion) interfering signals in the chromatograms and the identical retention times of the analyte in standard solutions and in extracts from samples in different columns, the procedure can be regarded specific for Phthalimide.

Linearity
The linearity of the method was studied with matrix matched standards between 3 ng/mL and 120 ng/mL (corresponding to 0.003 to 0.120 in mg/kg). The linear correlation coefficients were > 0.990, showing a good linearity. Plots of the graphs and parameter of the equations are available.

Accuracy
For quantification the samples are fortified at 0.01 and 0.1 mg/kg for both analytes. For confirmation only a fortification at 0.01 mg/kg is presented (both fortifications are determined in the ILV). 5 recoveries per concentration are determined. Mean recovery is between 70 and 120 %.

Repeatability
The relative standard deviation (RSD) over all fortification levels is within guideline requirements of RSD (< 20% for 0.1 mg/kg and < 30% for 0.01 mg/kg).

Matrix effects
Matrix effects were found to be significant (> ± 20%). Matrix matched standards were used for quantification for all matrices, by default.

LOQ: 0.01 mg/kg in all matrices.

Table A 24: Recovery results from method validation of phthalimide using the analytical method

Matrix	Analyte	Fortification level (mg/kg) (*n* = 5)	Mean recovery (%)	RSD (%)	Comments
Eggs	Phthalimide	0.01	112.0	2.6	*Quantification* Column: BEH C18 *m/z* 146.0 → 41.9
		0.1	103.7	3.2	
		0.01	107.8	0.9	*Confirmation* Column: ZORBAX SB-C3 *m/z* 146.0 → 41.9
Meat	Phthalimide	0.01	104.4	3.9	*Quantification* Column: BEH C18 *m/z* 146.0 → 41.9
		0.10	96.9	3.8	
		0.01	101.1	9.8	*Confirmation* Column: ZORBAX SB-C3 *m/z* 146.0 → 41.9
Fat	Phthalimide	0.01	114.3	3.6	*Quantification* Column: BEH C18 *m/z* 146.0 → 41.9
		0.10	108.1	3.1	
		0.01	104.6	2.6	*Confirmation* Column: ZORBAX SB-C3 *m/z* 146.0 → 41.9
Liver	Phthalimide	0.01	82.3	5.7	*Quantification* Column: BEH C18 *m/z* 146.0 → 41.9
		0.10	85.4	3.3	
		0.01	84.7	4.1	*Confirmation* Column: ZORBAX SB-C3 *m/z* 146.0 → 41.9
Milk	Phthalimide	0.01	82.6%	6.4%	*Quantification* Column: BEH C18 *m/z* 146.0 → 41.9
		0.10	83.8%	10.2%	
		0.01	101.2%	8.1%	*Confirmation* Column: ZORBAX SB-C3 *m/z* 146.0 → 41.9

Table A 25: Characteristics for the analytical method used for validation of folpet residues in animal matrices

	Phthalimide	
Specificity	**Primary method** LC – MS/MS Column: BEH C18 *m/z* 146.0 → 41.9 blank value < 30 % LOQ	**Confirmatory method** LC – MS/MS Column: ZORBAX SB-C3 m/z 146.0 → 41.9 blank value < 30 % LOQ

	Phthalimide	
Calibration (type, number of data points)	Eggs: C=8.3630E-04xS-0.30 (r=0.99952) Meat: C=7.1434E-04xS-0.72 (r=0.99996) Fat: C=7.7537E-04xS+0.16 (r=0.99978) Liver: C=8.6096E-04xS-0.25 (r=0.99979) 8 data points	Eggs: C=8.0830E-04xS+1.66 (r=0.99974) Meat: C=5.7436E-04xS-0.31 (r=0.99909) Fat: C=6.5612E-04xS+0.42 (r=0.99870) Liver: C=6.5978E-04xS+0.83 (r=0.99927) 8 data points
Calibration range	Accepted calibration range in concentration units: 3-120 ng/ml Corresponding calibration range in mass ratio units for the sample: 0.003-0.120 mg/kg	
Assessment of matrix effects is presented	Yes Matrix effects were found to be significant (> ± 20%). Matrix matched standards were used for quantification for all matrices	
Limit of determination/quantification	LOQ= 0.01 mg/kg LOD= 0.003 mg/kg	

Conclusion
The method was found to be valid according to the guidance documents SANCO/825/00 rev 8.1 for the determination of phthalimide with both primary and confirmatory method in liver, meat, fat, and eggs at a LOQ of 0.01 mg/kg. All parameters are also according to the new guidance SANTE/2020/12830 rev. 2.
Due to low recoveries obtained, the method for the analysis of phthalimide expressed as folpet could not be successfully validated, with both primary and confirmatory method, for milk, according to the guidance document SANCO/825/00 rev 8.1 with a LOQ of 0.01 mg/kg. Therefore, the method is not appropriate for the determination of this analyte in milk.
Since the method from report No. R B4281 could not be validated by an independent laboratory for the determination of phthalimide in milk, the multi-residue method DFG S19 was additionally validated for this matrix. In addition, further animal matrices (eggs and fat) were tested with the DFG S19 method.

A 2.2.2.2.1.1 Independent laboratory validation

Comments of zRMS:	The method was evaluated by zRMS-Greece in RR of SAP50SCF (December 2023). zRMS-Greece comments: *The method is considered acceptable.* zRMS-PL remark: The method is not appropriate for the determination of phthalimide in milk.

Reference:	KCP 5.2/10
Report	Independent Laboratory Validation of the Analytical Method for the Determination of Phthalimide in Animal Matrices by LC-MS/MS. Meseguer, 2016, Report no: S14-05780
Guideline(s):	SANCO/825/00 rev. 8.1 OECD ENV/JM/MONO(2007)17 OPPTS 860.1340
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods
Test item
muscle, liver, milk

Analyte
Phthalimide

Results and discussions
An independent laboratory validation was conducted for liver, meat, and milk matrices. The analytical method is the same used in study RF B4281.
The method was found to be valid according to the guidance documents SANCO/825/00 rev 8.1 for the determination of phthalimide with both primary and confirmatory method in liver and meat with a LOQ of 0.01 mg/kg.
Due to low recoveries obtained, the method for the analysis of both analytes with both primary and confirmatory method in milk could not be successfully validated according to the guidance document SANCO/825/00 rev 8.1 with a LOQ of 0.01 mg/kg.

Table A 26: Recovery results from independent laboratory validation of phthalimide using the analytical method

Matrix	Analyte	Fortification level (mg/kg) (*n* = 5)	Mean recovery (%)	RSD (%)	Comments
Liver	Phthalimide	0.01	90	3	*Quantification* Column: BEH C18 *m/z* 146.0 → 41.9
		0.1*	82	8	
		0.01	90	4	*Confirmation* Column: ZORBAX SB-C3 *m/z* 146.0 → 41.9
		0.1	83	6	
Muscle	Phthalimide	0.01	86	3	*Quantification* Column: BEH C18 *m/z* 146.0 → 41.9
		0.1	88	4	
		0.01	87	3	*Confirmation* Column: ZORBAX SB-C3 *m/z* 146.0 → 41.9
		0.1	86	4	

* The Dixon test was performed, and one value (8%) was identified as an outlier. The mean recovery and the RSD were obtained for n=4.

Table A 27: Characteristics for the analytical method used for independent laboratory validation of folpet residues in animal matrices

	Phthalimide	
Specificity	**Primary method** LC-MS/MS *m/z* 146.0 → 41.9 (Column: BEH C18) blank value < 30 % LOQ	**Confirmatory method** LC-MS/MS *m/z* 146.0 → 41.9 (Column: ZORBAX SB-C3) blank value < 30 % LOQ
Calibration (type, number of data points)	Muscle: y=895x+149 (r^2=0.9948) 7 data points Liver: y=506x-670 (r^2=0.9992)	Muscle: y=1720x+37 (r^2=0.9986) 8 data points Liver: y=1419x+1268 (r^2=0.9994)

	Phthalimide	
	8 data points	8 data points
Calibration range	Accepted calibration range in concentration units: 3 – 120 ng/mL Corresponding calibration range in mass ratio units for the sample: 0.003 – 0.12 mg/kg	
Assessment of matrix effects is presented	Yes	
Limit of determination/quantification	LOQ = 0.01 mg/kg LOD = 0.003 mg/kg	

Conclusion
The method was found to be valid according to the guidance documents SANCO/825/00 rev 8.1 for the determination of phthalimide with both primary and confirmatory method in liver and muscle at a LOQ of 0.01 mg/kg. All parameters are also according to the new guidance SANTE/2020/12830 rev. 2.
Due to low recoveries obtained, the method for the analysis of phthalimide with both primary and confirmatory method in milk could not be successfully validated according to the guidance document SANCO/825/00 rev 8.1 with a LOQ of 0.01 mg/kg. Therefore, the method is not appropriate for the determination of phthalimide in milk.

A 2.2.2.2.2 Analytical method 2

Comments of zRMS:	The method was evaluated by zRMS-Greece in RR of SAP50SCF (December 2023). zRMS-Greece comments: *The method is considered acceptable.*

Reference:	KCP 5.2/07
Report	Validation of the multi-residue method DFG-S19 for the determination of phthalimide in milk, fat, and eggs. Wiesner, F., Breyer, N., Trümper, C., 2016, Report no: S16-00672 (BEL-1602V)
Guideline(s):	SANCO/825/00 rev. 8.1 SANCO/3029/99 rev. 4
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods
Test item
Milk, eggs, fat

Analyte
Phthalimide

Principle of method
Samples of milk and egg were extracted with acetone according to multi-residue method DFG S19 module E1. Before the addition of acetone, warm water was added in an amount that takes full account of the natural water content of the specimen - so that the acetone/water ratio during extraction is 2/1 (v/v). For liquid-liquid partition, ethyl acetate/cyclohexane (1/1, v/v) and sodium chloride were added, and after repeated mixing excess water was separated. An aliquot of the organic phase was evaporated to a watery residue.
Samples of fat were dissolved in a mixture of ethyl acetate/cyclohexane (1/1, v/v) according to multi-residue method DFG S19 module E6.

The residues obtained from extraction module E1 and E6 were cleaned up by gel permeation chromatography on Bio Beads S-X3 polystyrene gel using a mixture of ethyl acetate/cyclohexane (1/1, v/v) as eluant. The fraction containing phthalimide residues was concentrated to dryness. After reconstitution in acetonitrile/1% acetic acid (3/7, v/v), the final extracts of milk, eggs and fat were analysed for phthalimide by liquid chromatography using tandem mass spectrometric detection (LC-MS/MS).

HPLC Conditions

Column: Develosil RP Aqueous-3 140A (150 x 3 mm; particle size 3 µm)
Mobile phases: water / methanol (both with 0.5% formic acid); gradient mode

MS/MS Conditions

Quantification: *m/z* 148 → 130
Confirmation: *m/z* 148 → 102

Results and discussions

Specificity/Interference
Due to the use of a highly specific detection system (MS/MS), the lack of significant (> 30 % of LOQ for the quantifier ion) interfering signals in the chromatograms and the identical retention times of the analyte in standard solutions and in extracts from samples, the procedure can be regarded to be highly specific for phthalimide.

Linearity
The linearity of the method was studied with external standards between 1.0 ng/mL and 200 ng/mL (corresponding to residue levels between 0.0021 to 0.43 mg/kg for milk and eggs and between 0.0025 to 0.50 mg/kg for fat). The linear correlation coefficients were > 0.99, showing a good linearity.
Plots of the graphs and parameter of the equations are available.

Accuracy
For quantification the samples are fortified at 0.01 and 0.1 mg/kg. 5 recoveries per concentration are determined.
Mean Recovery is between 70 and 120%.

Repeatability
The relative standard deviation (RSD) over all fortification levels is within guideline requirements of RSD (< 20% for 0.1 mg/kg and < 30% for 0.01 mg/kg).

Matrix effects
Matrix effects were found to be insignificant (> ± 20%) in the primary study. Therefore, solvent standards were used for quantification for all matrices. In the ILV study the matrix effect was significant in fat.

LOQ: 0.01 mg/kg

Table A 28: Recovery results from method validation of phthalimide using the analytical method

Matrix	Analyte	Fortification level (mg/kg) (*n* = 5)	Mean recovery (%)	RSD (%)	Comments
Milk	Phthalimide	0.01	97	3.1	*Quantification* *m/z* 148 → 130
		0.10	98	2.4	
		0.01	99	6.2	*Confirmation* *m/z* 148 → 102
		0.10	99	2.1	
Egg	Phthalimide	0.01	98	4.5	*Quantification*

Matrix	Analyte	Fortification level (mg/kg) (n = 5)	Mean recovery (%)	RSD (%)	Comments
		0.1	91	4.2	*m/z* 148 → 130
		0.01	96	3.1	*Confirmation* *m/z* 148 → 102
		0.1	90	4.4	
Fat	Phthalimide	0.01	105	3.9	*Quantification* *m/z* 148 → 130
		0.10	85	12	
		0.01	107	4.1	*Confirmation* *m/z* 148 → 102
		0.10	85	11	

Table A 29: Characteristics for the analytical method used for validation of folpet residues in animal matrices

	Phthalimide	
Specificity	**Primary method** LC – MS/MS *m/z* 148 → 130 blank value < 30 % LOQ	**Confirmatory method** LC – MS/MS *m/z* 148 → 102 blank value < 30 % LOQ
Calibration (type, number of data points)	Milk: y=30383.6164x+71732.0083 (r=0.9994) Fat: y=38568.9467x-42230.4916 (r=0.9997) Egg: y=39609.6689x+13366.8278 (r=0.9999) 7 data points	Milk: y=19134.7160x+41571.3771 (r=0.9994) Fat: y=24129.5851x-36288.5510 (r=0.9996) Egg: y=24884.0487x+17651.1391 (r=0.9998) 7 data points
Calibration range	Accepted calibration range in concentration units: 1 – 200 ng/mL Corresponding calibration range in mass ratio units for the sample: Milk and eggs: 0.0021 – 0.43 mg/kg Fat: 0.0025 – 0.50 mg/kg	
Assessment of matrix effects is presented	Yes	
Limit of determination/quantification	LOQ= 0.01 mg/kg LOD= 0.003 mg/kg	

Conclusion
The multi-residue method DFG S19 was successfully validated by an independent laboratory for the analysis of phthalimide fat, milk, and eggs at the tested LOQ of 0.01 mg/kg according to the guidance document SANCO/825/00 rev. 8.1. Moreover, this method is also valid according to the new guidance SANTE/2020/12830 rev.2.

A 2.2.2.2.2.1 Independent laboratory validation

Comments of zRMS:	The method was evaluated by zRMS-Greece in RR of SAP50SCF (December 2023). zRMS-Greece comments: *The study is considered acceptable.*

Reference: KCP 5.2/08

Report	Independent Laboratory Validation of an analytical method for the determination of phthalimide in milk, eggs, and fat. Mewis, A., 2016, Report no: S16-00717
Guideline(s):	SANCO/825/00 rev. 8.1 SANCO/3029/99 rev. 4
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

Test item

Milk, fat, and eggs.

Analyte

Phthalimide

Results and discussions

An independent laboratory validation was conducted for all three matrices. Analysis of samples was performed and detected according to the primary method differing slightly in calibration range but still in line with SANCO/825/00 rev 8.1.

Table A 30: Recovery results from independent laboratory validation of phthalimide using the analytical method

Matrix	Analyte	Fortification level (mg/kg) (*n* = 5)	Mean recovery (%)	RSD (%)	Comments
Fat	Phthalimide	0.01	88	19	*Quantification* *m/z* 148 → 130
		0.1	97	3	
		0.01	91	18	*Confirmation* *m/z* 148 → 102
		0.1	97	4	
Eggs	Phthalimide	0.01	80	5	*Quantification* *m/z* 148 → 130
		0.1	78	4	
		0.01	89	11	*Confirmation* *m/z* 148 → 102
		0.1	78	5	
Milk	Phthalimide	0.01	86	5	*Quantification* *m/z* 148 → 130
		0.1	86	8	
		0.01	83	9	*Confirmation* *m/z* 148 → 102
		0.1	85	11	

Table A 31: Characteristics for the analytical method used for independent laboratory validation of folpet residues in animal matrices

	Phthalimide	
Specificity	**Primary method** LC – MS/MS *m/z* 148 → 130 blank value < 30 % LOQ	**Confirmatory method** LC – MS/MS *m/z* 148 → 102 blank value < 30 % LOQ
Calibration (type, number of data points)	Fat: y=9.6e+004x+4.44e+003 (r=0.9997)	Fat: y=6.26e+004x+1.06e+004 (r=0.9998)

	Phthalimide	
	Eggs: y=9.28e+004x+3.18e+004 (r=0.9990) Milk: y=9.77e+004x+3.93e+004 (r=0.9998) 6 data points	Eggs: y=6.07e+004x+5.69e+004 (r=0.9996) Milk: y=6.53e+004x+5.31e+004 (r=0.9998) 6 data points
Calibration range	Accepted calibration range in concentration units: 3 – 120 ng/mL Corresponding calibration range in mass ratio units for the sample: 0.003 – 0.12 mg/kg	
Assessment of matrix effects is presented	Yes	
Limit of determination/quantification	LOQ = 0.01 mg/kg LOD = 0.003 mg/kg	

Conclusion
The multi-residue method DFG S19 was successfully validated by an independent laboratory for the analysis of phthalimide in fat, milk, and eggs at the tested LOQ of 0.01 mg/kg according to the guidance document SANCO/825/00 rev. 8.1. Moreover, this method is also valid according to the new guidance SANTE/2020/12830 rev.2.

A 2.2.2.2.2.2 Extraction efficiency

New data has not been provided.

A 2.2.2.3 Description of analytical methods for the analysis of soil (KCP 5.2)

A 2.2.2.3.1 Analytical method

Comments of zRMS:	The method was evaluated by zRMS-Greece in RR of SAP50SCF (December 2023). zRMS-Greece comments: *The study is considered acceptable.*

Reference:	KCP 5.2/11
Report	Validation of the analytical method for the determination of folpet in soil, Schlewitz, P., 2015b, Report no: R B4282
Guideline(s):	SANCO/825/00 rev. 8.1 SANCO/3029/99 rev. 4
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

Test item

Matrix	Description/origin
Soil	Soil for truck farming
Soil texture (USDA)	Sandy loam (10.5% clay, 37.8% silt, 51.8% sand)
Soil pH (H_2O)	7.0
organic carbon content (% OC)	1.12

Analyte
Folpet

Principle of method
Homogenised soil (approximately 10 g) is extracted with ethyl acetate and concentrated o-phosphoric acid in the presence of magnesium sulphate and sodium chloride. Folpet is determined by liquid-chromatography with MS/MS detector.

HPLC Conditions

Quantification: Column: BEH C18 (2.1 x 50 mm; particle size 1.7 µm)
Mobile phases: water / methanol; gradient mode

Confirmation: Column: ZORBAX SB-C3 (3 x 150 mm; particle size 5 µm)
Mobile phases: water / methanol; gradient mode

MS/MS Conditions

Quantification & Confirmation: m/z 146 → 41.9

Results and discussions
Specificity/Interference

Due to the use of a highly specific detection system (MS/MS), the lack of significant (> 30 % of LOQ for the quantifier ion) interfering signals in the chromatograms and the identical retention times of the analyte in standard solutions and in extracts from samples, the procedure can be regarded to be highly specific for Folpet.

Linearity

The linearity of the method was studied with matrix matched standards between 3 ng/mL and 120 ng/mL (corresponding to 0.003 to 0.120 in mg/kg). The linear correlation coefficients were > 0.990, showing a good linearity. Plots of the graphs and parameter of the equations are available.

Accuracy

For quantification the samples are fortified at 0.01 and 0.1 mg/kg for both analytes. For confirmation only a fortification at 0.01 mg/kg is presented. 5 recoveries per concentration are determined. Mean Recovery is between 70 and 120 %.

Repeatability

The relative standard deviation (RSD) over all fortification levels is within guideline requirements of RSD (< 20% for 0.1 mg/kg and < 30% for 0.01 mg/kg).

Matrix effects

Matrix effects were found to be significant (> ± 20%). Matrix matched standards were used for quantification for all matrices, by default.

LOQ: 0.01 mg/kg in all matrices.

Table A 32: Recovery results from method validation of folpet using the analytical method

Matrix	Analyte	Fortification level (mg/kg) (*n* = 5)	Mean recovery (%)	RSD (%)	Comments
Soil	Folpet	0.01	77.7	9.4	*Quantification* Column: BEH C18 *m/z* 146.0 → 41.9
		0.10	87.2	12.0	
		0.01	85.2	10.9	*Confirmation* Column: ZORBAX SB-C3 *m/z* 146.0 → 41.9

Table A 33: Characteristics for the analytical method used for validation of folpet residues in soil

	~~Phthalimide~~ **Folpet**	
Specificity	**Primary method** LC – MS/MS Column: BEH C18 *m/z* 146.0 → 41.9	**Confirmatory method** LC – MS/MS Column: ZORBAX SB-C3 *m/z* 146.0 → 41.9
Calibration (type, number of data points)	C=1.1829E-02xS + 1.00 (r=0.99729) 8 data points	C=9.5800E-03xS – 0.64 (r=0.99982) 8 data points
Calibration range	Accepted calibration range in concentration units: 3 – 120 ng/ml Corresponding calibration range in mass ratio units for the sample: 0.003 – 0.12 mg/kg	
Assessment of matrix effects is presented	Yes	
Limit of determination/quantification	LOQ= 0.01 mg/kg LOD= 0.003 mg/kg	

Conclusion
The method was found to be valid according to the guidance documents SANCO/825/00 rev 8.1 for the determination of folpet with both primary and confirmatory method in soil at a LOQ of 0.01 mg/kg. Furthermore, the method is also valid according to the new guidance SANTE/2020/12830 rev.2.

A 2.2.2.4 Description of Methods for the Analysis of Water (KCP 5.2)

A 2.2.2.4.1 Analytical method 1

Comments of zRMS:	The method was evaluated by zRMS-Greece in RR of SAP50SCF (December 2023). zRMS-Greece comments: *The GC-MS analytical method is acceptable and validated for the determination of folpet and phthalimide in drinking water.*

Reference:	KCP 5.2/12
Report	Folpet and phthalimide: Validation of Methodology for the Determination of Residues of Folpet and Phthalimide in Drinking Water. Aris, D., 2011, Report no: ZEF0005
Guideline(s):	SANCO/3029/99 rev.4 SANCO/825/00 rev. 8.1
Deviations:	No
GLP:	Yes

Acceptability: Yes

Materials and methods
Test item
Drinking water

Analyte
Folpet
Phthalimide

Principle of method
For folpet, the method comprised of extraction by liquid:liquid partition with toluene. For phthalimide, the method comprised of extraction by liquid:liquid partition with dichloromethane. For both folpet and phthalimide quantitation was performed using gas chromatography with mass spectrometric detection (GC MS). Two GC columns were used, one for quantitation and the other for confirmation purposes.

GC Conditions

Quantification: Optima-17 (30 m x 0.25 mm x 0.5 µm film thickness); He
Confirmation: DB-5 (30 m x 0.25 mm x 0.25 µm film thickness); He

MS Conditions

m/z 146 – Folpet
m/z 147 – Phthalimide

Results and discussions
Specificity/Interference
Due to the use of a highly specific detection system (MS), the lack of significant (> 30 % of LOQ for the quantifier ion) interfering signals in the chromatograms and the identical retention times of the analyte in standard solutions and in extracts from samples, the procedure can be regarded to be highly specific for folpet and phthalimide.
For confirmation a column of a different polarity was used for folpet and phthalimide.

Linearity
The linearity of the method was studied with matrix matched/external standards between 0.1 µg/L and 10 µg/L (equivalent to 0.025 to 2.5 µg/L in matrix). The linear correlation coefficients were > 0.99 (except for phthalimide using the DB-5 confirmation column which gave a quadratic response with good coefficient), showing a good linearity.
Plots of the graphs and parameter of the equations are available.

Accuracy
For quantification, the samples are fortified at 0.1 and 1 µg/L. 5 recoveries per concentration are determined.
Mean recovery is between 70 and 120 %.

Repeatability
The relative standard deviation (RSD) over all fortification levels is within guideline requirements of RSD (< 30% for 0.1 µg/L)

LOQ: 0.1 µg/L for all analytes

Table A 34: Recovery results from method validation of folpet and phthalimide using the analytical method

Matrix	Analyte	Fortification level (µg/L) (*n* = 5)	Mean recovery (%)	RSD (%)	Comments
Drinking water	Folpet	0.1	90.2	3.5	*Quantification* GC-MS, column: Optima-17 *m/z* 146
		1	99.0	8.3	
		0.1	104.2	5.4	*Confirmation* GC-MS, column: DB-5 *m/z* 146
		1	97.0	3.9	
	Phthalimide	0.1	74.2	2.2	*Quantification* GC-MS, column: Optima-17 *m/z* 147
		1	74.6	4.1	
		0.1	82.6	7.7	*Confirmation* GC-MS, column: DB-5 *m/z* 147
		1	76.4	7.1	

Table A 35: Characteristics for the analytical method used for validation of folpet residues in water

	Folpet	Phthalimide
Specificity	Primary method: GC-MS, column: Optima-17 *m/z* 146 Confirmatory method: GC-MS, column: DB-5 *m/z* 146	Primary method: GC-MS, column: Optima-17 *m/z* 147 Confirmatory method: GC-MS, column: DB-5 *m/z* 147
Calibration (type, number of data points)	Primary method: y=639.079x – 59.3437 (r^2=0.997297) Confirmatory method: y=1091.88x – 67.0727 (r^2=0.999768) 8 data points	Primary method: y=15522.1x + 2825.76 (r^2=0.998138) Confirmatory method: y=242.743x^2 + 2844.90x + 378.864 (r^2=0.999583) 8 data points
Calibration range	Accepted calibration range in concentration units: 1 – 10 ng/mL Corresponding calibration range in mass ratio units for the sample: 0.025 to 2.5 µg/L	Accepted calibration range in concentration units: 1 – 10 ng/mL Corresponding calibration range in mass ratio units for the sample: 0.025 to 2.5 µg/L
Assessment of matrix effects is presented	yes	yes
Limit of determination/quantification	LOQ=0.1 µg/L (equivalent to 0.4 ng/mL in the final extract) LOD=0.1 ng/L (equivalent to 0.025 µg/mL in sample matrix)	LOQ=0.1 µg/L (equivalent to 0.4 ng/mL in the final extract) LOD=0.1 ng/L (equivalent to 0.025 µg/mL in sample matrix)

Conclusion

The analytical method has been fully validated according to the guidance documents SANCO/825/00 rev. 8.1 for the determination of folpet and phthalimide at 0.1 and 1 µg/L in drinking water using gas chromatography with mass detection (GC-MS). The limit of quantitation (LOQ) of 0.1 µg/L of the residue method in this sample type was determined as the lowest level validated. All parameters are also according to the new guidance SANTE/2020/12830 rev. 2.

A 2.2.2.4.1.1 Independent laboratory validation

Comments of zRMS:	The method was evaluated by zRMS-Greece in RR of SAP50SCF (December 2023). zRMS-Greece comments: *The method is considered acceptable.*

Reference:	KCP 5.2/13
Report	Independent Laboratory Validation (ILV) of Analytical Methods for the Determination of Folpet and of Phthalimide in Water. Maas, X., Bendig, P., 2015, Report no: P 3812 G
Guideline(s):	SANCO/3029/99 rev.4 SANCO/825/00 rev. 8.1
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods
Test item
Surface water

Analyte
Folpet
Phthalimide

Principle of method
For folpet, the method comprised of extraction by liquid:liquid partition with toluene. For phthalimide, the method comprised of extraction by liquid:liquid partition with dichloromethane. For both folpet and phthalimide quantitation was performed using gas chromatography with mass spectrometric detection (GC MS). Two GC columns were used, one for quantitation and the other for confirmation purposes.

GC Conditions
Quantification: DB-17MS (30 m x 0.25 mm x 0.25 µm film thickness); He
Confirmation: Optima 5 HT (30 m x 0.25 mm x 0.25 µm film thickness); He

MS Conditions
m/z 146 – Folpet
m/z 147 – Phthalimide

Results and discussions
An independent laboratory validation was conducted. Surface water was used for this analysis. Analysis of samples was performed and detected according to the primary method with minor deviations (column of a different manufacturer was used, calibration range slightly different). The method was found to be valid according to the guidance documents SANCO/825/00 rev 8.1 and SANCO/3029/99 rev.4 for the determination of folpet and phthalimide with a LOQ of 0.1 µg/L.

Table A 36: Recovery results from method validation of folpet and phthalimide using the analytical method

Matrix	Analyte	Fortification level (µg/L) (*n* = 5)	Mean recovery (%)	RSD (%)	Comments
Surface water	Folpet	0.10	84	5	*Quantification*

Matrix	Analyte	Fortification level (µg/L) (*n* = 5)	Mean recovery (%)	RSD (%)	Comments
		1.0	84	11	GC-MS, column: DB-17MS *m/z* 146
		0.10	78	5	*Confirmation* GC-MS, column: Optima 5 HT *m/z* 146
		1.0	86	13	
	Phthalimide	0.10	90	8	*Quantification* GC-MS, column: DB-17MS *m/z* 147
		1.0	79	9	
		0.10	82	7	*Confirmation* GC-MS, column: Optima 5 HT *m/z* 147
		1.0	72	7	

Table A 37: Characteristics for the analytical method used for validation of folpet and phthalimide residues in water

	Folpet	Phthalimide
Specificity	Primary method: GC-MS, column: DB-17MS *m/z* 146 Confirmatory method: GC-MS, column: Optima 5 HT *m/z* 146	Primary method: GC-MS, column: DB-17MS *m/z* 147 Confirmatory method: GC-MS, column: Optima 5 HT *m/z* 147
Calibration (type, number of data points)	Primary method: $y=72909.6x – 1456.03$ ($r^2=0.9918$) 8 data points Confirmatory method: $y=4174.23x^2 + 16306.8x – 538.755$ ($r^2=0.9931$) 7 data points	Primary method: $y=2.11101e+006x + 574314$ ($r^2=0.9977$) 9 data points Confirmatory method: $y= – 28223.3x^2 + 1.4949e+006x + 237743$ ($r^2=0.9915$) 8 data points
Calibration range	Accepted calibration range in concentration units: 1 – 10 ng/mL Corresponding calibration range in mass ratio units for the sample: 0.025 – 2.5 µg/L	Accepted calibration range in concentration units: 1 – 10 ng/mL Corresponding calibration range in mass ratio units for the sample: 0.025 – 2.5 µg/L
Assessment of matrix effects is presented	yes	yes
Limit of determination/quantification	LOQ=0.1 µg/L LOD ≤ 0.025 µg/mL	LOQ=0.1 µg/L LOD ≤ 0.025 µg/mL

Conclusion

The independent laboratory validation (ILV) for the determination of folpet residues in water by GC/MS, demonstrates a LOQ of 0.1 µg/L and a limit of detection (LOD) of ≤ 0.025 µg/L. The ILV was performed in surface water (original method used drinking water) and is thus representing a successful validation for this matrix type according to EC guidance documents SANCO/825/00 rev. 8.1 and SANCO/3029/99 rev.4. It is concluded that the methods described in the original validation report were applicable and served its original purpose. All parameters are also according to the new guidance SANTE/2020/12830 rev. 2.

A 2.2.2.5 Description of Methods for the Analysis of Air (KCP 5.2)

Comments of zRMS:	The method was evaluated by zRMS-Greece in RR of SAP50SCF (December 2023).

	zRMS-Greece comments: *The GC-MS analytical method is acceptable and validated for the determination of folpet in air.*

Reference:	KCP 5.2/14
Report	Folpet and phthalimide: Validation of Methodology for the Determination of Residues of Folpet and Phthalimide in Air. Aris, D., 2012, Report no: ZEF0006
Guideline(s):	SANCO/3029/99 rev.4 SANCO/825/00 rev. 8.1 of November 2010
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods
Test item
Air
(Temperature = 35 °C ± 0.8 ºC, relative humidity = 80% ± 2.1%)

Analyte
Folpet
Phthalimide

Principle of method
For folpet, air cartridges were extracted with acetonitrile and diluted with 2% diglyme in toluene. For phthalimide, air cartridges were extracted with acetonitrile and diluted with 2% diglyme in dichloromethane. For both folpet and phthalimide quantitation was performed using gas chromatography with mass spectrometric detection (GC MS).

GC Conditions:

Quantification: Optima-17 (30 m x 0.25 mm x 0.5 µm film thickness); He

MS Conditions:

m/z 146 – Folpet
m/z 147 – Phthalimide

Results and discussions
Specificity/Interference
Control (untreated) samples of the sorbent material (Tenax) were analysed using the analytical method. There was no apparent response (i.e. < 30 % of the LOQ) in the region of the chromatograms corresponding to the retention time of folpet or phthalimide.

Linearity
The linearity of the method was studied with matrix matched standards between 0.1 and 10 ng/mL (equivalent to 5.56 to 556 µg/m³). The correlation coefficients were > 0.99, showing a good linearity.
Plots of the graphs and parameter of the equations are available.

Accuracy
For quantification the samples are fortified at 30 and 300 µg/m³ (equivalent to 10.8 µg and 108 µg on sorbent material). 5 recoveries per concentration are determined. No breakthrough was observed on any of the samples.

Mean recovery is between 70 and 110 %.

Repeatability
The relative standard deviation (RSD) over all fortification levels is within guideline requirements of RSD (< 20%).

Matrix effects
No significant matrix effects. Solvent standards were used.

LOQ: 30 µg/m³ (equivalent to 10.8 µg on sorbent material)

Table A 38: Recovery results from method validation of folpet and phthalimide using the analytical method

Matrix	Analyte	Fortification level (µg) (*n* = 5)	Mean recovery (%)	RSD (%)	Comments
Air	Folpet	10.8	105	2.2	GC-MS, column: Optima-17 *m/z* 146
		108	96	3.0	
	Phthalimide	10.8	102	6.7	GC-MS, column: Optima-17 *m/z* 147
		108	98	5.4	

Table A 39: Characteristics for the analytical method used for validation of folpet and phthalimide residues in air

	Folpet	Phthalimide
Specificity	GC-MS, column: Optima-17 *m/z* 146	GC-MS, column: Optima-17 *m/z* 147
Calibration (type, number of data points)	y=1465.87x – 55.2225 (r=0.999501) 9 data points	y=17468.1x + 3145.71 (r=0.999523) 9 data points
Calibration range	Accepted calibration range in concentration units: 0.1 – 10 ng/mL Corresponding calibration range in mass ratio units for the sample: 5.56 to 556 µg/m³	Accepted calibration range in concentration units: 0.1 – 10 ng/mL Corresponding calibration range in mass ratio units for the sample: 5.56 to 556 µg/m³
Assessment of matrix effects is presented	yes	yes
Limit of determination/quantification	LOQ=30 µg/m³ (equivalent to 10.8 µg on sorbent material) LOD=0.1 ng/L (equivalent to 5.56 µg/mL in matrix)	LOQ=30 µg/m³ (equivalent to 10.8 µg on sorbent material) LOD=0.1 ng/L (equivalent to 5.56 µg/mL in matrix)

Conclusion
For confirmatory purposes it was also demonstrated in the study ZEF0005, submitted in KCP 5.2/12 (Folpet and Phthalimide: Validation of methodology for the determination of residues of folpet and phthalimide in drinking water; Report no: ZEF0005) that a second analytical column could be successfully used with a different stationary phase for this purpose. The quantitation column used in both studies was the medium polar Optima-17 (50% phenyl – 50% methylpolysiloxane) and the confirmatory column demonstrated as suitable in study ZEF0005 was the non-polar DB-5 (5% phenyl – 95% methylpolysiloxane). Therefore, according to the Regulatory Guideline SANCO/825/00 rev. 8.1 Section 7.7, no further confirmation was

required in this study and the method was find valid for the determination of folpet and phthalimide. All parameters are also according to the new guidance SANTE/2020/12830 rev. 2.

A 2.2.2.6 Description of Methods for the Analysis of Body Fluids and Tissues (KCP 5.2)

A 2.2.2.6.1 Analytical method 1

Comments of zRMS:	The method was evaluated by zRMS-Greece in RR of SAP50SCF (December 2023). zRMS-Greece comments: *The LC-MS/MS analytical method for monitoring of phthalimide residues in body fluids (urine) is considered validated in terms of linearity, specificity, precision and accuracy, with LOQ 0.05 mg/L.* *However, according to GD SANTE/2020/12830-rev.1, which is now in force, the LOQ shall be at 0.01 mg/L for body fluids.* *Therefore, a data gap is proposed for a lower LOQ of 0.01 mg/L in accordance to the GD. Any further data should be addressed at active substance level.*

Reference:	KCP 5.2/15
Report	Validation of the multi-residue method DFG S19 for the determination of phthalimide in urine. Wiesner, F., Breyer, N., 2016, Report no: S16-02058 (BEL-1603V)
Guideline(s):	SANCO/825/00 rev. 8.1 (2010) SANCO/3029/99 rev. 4 (2000)
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods
Test item
Urine

Analyte
Phthalimide

Principle of method
The urine samples were extracted with acetone according to multi-residue method DFG S19 module E1. The final extracts were analysed for phthalimide by liquid chromatography with MS/MS detection.

HPLC Conditions

Column: Phenomenex Develosil RP Aqueous-3 (3 x 150 mm; particle size 3 µm)
Mobile phases: water / methanol; gradient mode

MS/MS Conditions

Quantification: *m/z* 148 → 130
Confirmation: *m/z* 148 → 102

Results and discussions
Specificity/Interference
Due to the use of a highly specific detection system (MS/MS), the lack of significant (> 30 % of LOQ for

the quantifier ion) interfering signals in the chromatograms and the identical retention times of the analyte in standard solutions and in extracts from samples, the procedure can be regarded to be highly specific for phthalimide.

Linearity
The linearity of the detector response was demonstrated by single determination of solvent calibration standards at concentration levels between 3.0 ng/L and 200 ng/L (corresponding to 0.013 to 0.86 mg/L in the matrix). The linear correlation coefficients were > 0.99, showing a good linearity.
Plots of the graphs and parameter of the equations are available.

Accuracy
For quantification the samples were fortified at 0.05 mg/L. 5 recoveries are determined.
Mean Recovery is between 70 and 120 %.

Repeatability
The relative standard deviation (RSD) over all fortification levels is within guideline requirements of RSD (< 20%)

Matrix effects
No significant matrix effects. Solvent standards were used for quantification.

LOQ: 0.05 mg/L

Table A 40: Recovery results from method validation of phthalimide using the analytical method

Matrix	Analyte	Fortification level (mg/L) (n = 5)	Mean recovery (%)	RSD (%)	Comments
Urine	Phthalimide	0.05	87	3.2	*Quantification* *m/z* 148 → 130
		0.05	83	6.2	*Confirmation* *m/z* 148 → 102

Table A 41: Characteristics for the analytical method used for validation of folpet residues in urine

	Phthalimide
Specificity	LC-MS/MS Primary method: *m/z* 148 → 130 Confirmatory method: *m/z* 148 → 102
Calibration (type, number of data points)	Primary method: y=41719.3082x – 18584.5107 (r=1.0000) Confirmatory method: y=25728.1308x – 14259.2504 (r=1.0000) 7 data points
Calibration range	Accepted calibration range in concentration units: 3.0 – 200 ng/mL Corresponding calibration range in mass ratio units for the sample: 0.013 – 0.86 mg/L
Assessment of matrix effects is presented	Yes
Limit of determination/quantification	LOQ=0.05 mg/L LOD=0.015 mg/L

Conclusion
The method was found to be valid according to the guidance documents SANCO/825/00 rev 8.1 for the determination of phthalimide in urine. Furthermore, the method is also valid according to the new guidance SANTE/2020/12830 rev.2.

A 2.2.2.7 A.2.A.9 Other Studies/ Information

No new or additional studies have been submitted.