

# REGISTRATION REPORT

## Part B

### Section 5

#### **Analytical Methods**

Detailed summary of the risk assessment

Product code: SAP50SFC

Product name(s): FOLPEC

Chemical active substance:

Folpet, 500 g/L

Central Zone

Zonal Rapporteur Member State: Poland

#### CORE ASSESSMENT

(authorization)

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

Submission date: December 2023

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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 (According Art. 33 of Regulation EC No 1107/2009)
April 2024	V1 – Updated version submitted by the Selectis Produtos para a Agricultura, S.A. answering Poland request in the frame of new PPP registration (According Art. 33 of Regulation EC No 1107/2009)
May 2024	V2 – Updated version to include the analytical phase report of the study KCP 10.2.1/05 at zRMS request. All changes are highlighted in green.
June 2024	<p>Initial assessment by the zRMS</p> <p>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 <b>highlighted in grey</b>. Not agreed or not relevant information are <del>struck through</del> and <b>shaded</b> for transparency.</p> <p>Following the evaluation and before sending the document for commenting, all coloured highlighting was removed, from the parts updated by the Applicant, for better legibility.</p>
August 2024	<p>Final report (Core Assessment updated following the commenting period)</p> <p>No additional information or assessments after the commenting period.</p>

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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:

- lower LOQ at 0.01 mg/L for phthalimide for body fluids. This data gap should be addressed at active substance level.

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 folpet in plant protection product is provided as follows:

Comments of zRMS:	Study acceptable. The analytical method for the determination of Folpet in FOLPEC was fully validated according to SANCO/3030/99 rev. 5.
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Reference:	KCP 5.1.1/01
Report	FOLPET 500 g/L SC (SAP50SCF): Physical, chemical and technical properties of the plant protection product, Morais, F., 2022, Report no EF/375/21 – Final Report – 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. Folpet content is quantified by an UPLC-PDA method and identify by GC-MS.

#### *Chromatographic conditions*

##### *· UPLC-PDA for active substance quantification*

<b>Mobile phase</b>	Acetonitrile: 0.25% ammonium acetate (50:50)
<b>Run time</b>	5 min
<b>Flow</b>	0.250 mL/min
<b>Column</b>	Acquity C18 UPLC BEH; 50 mm × 2.1 mm; 1.7 µm
<b>Column temperature</b>	30 °C
<b>Detection wavelength</b>	225 nm
<b>Injection volume</b>	0.1 µL
<b>Retention time</b>	Around 2.4 minutes

##### *· GC-MS conditions for active substance identification*

<b>Injector method</b>	<i>Injection volume</i>	1.00 µL
	<i>Pre-inj dwell time</i>	3 s
	<i>Post-inj dwell time</i>	3 s
<b>GC method</b>	<i>Temperature</i>	80 °C 15.0 °C/min until 250 °C (maintain for 8.67 minutes)
	<i>Column</i>	TG-5MS, 30 m × 0.25 mm × 0.25 µm film thickness
	<i>S/SL mode</i>	Split
	<i>Inlet temperature</i>	225 °C
	<i>Split flow</i>	60 mL/min (constant flow)
	<i>Carrier flow</i>	1.500 mL/min
<b>Detector Method (MS/MS)</b>	<i>Temperature</i>	280 °C
	<i>Ion source</i>	230 °C

	<i>Start time</i>	2 minutes
	<i>Ionization mode</i>	EI
	<i>Ion polarity</i>	Positive
	<i>Acquisition mode</i>	SCAN (100 amu – 600 amu)
<b>Retention times</b>	<i>PMM</i>	Around 11.5 minutes

#### Standard solution preparation

Weigh, in duplicate, about 50 mg  $\pm$  10% of folpet reference material into a 25 mL volumetric flask. Dissolve and complete the volume with acetonitrile (2.0 mg/mL). From this solution transfer 1.0 mL into a 10 mL volumetric flask and complete the volume with acetonitrile (0.2 mg/mL).

#### Sample solution preparation

Weigh approximately 49 mg  $\pm$  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  $\mu$ m disk filter (0.49 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 SAP50SCF**

	<b>Folpet</b>
<b>Author(s), year</b>	Morais, F., 2022
<b>Principle of method</b>	UPLC-PDA
<b>Linearity</b> (linear between mg/L / % range of the declared content) (correlation coefficient, expressed as r)	Range: [7.56% - 189.85%], [0.0371 mg/mL – 0.9303 mg/mL]  $y = 77993.6503x - 68.6572$ $r = 0.9998$
<b>Precision – Repeatability Mean</b> <b>n = 5</b> (%RSD)	System repeatability: RSD = 1.10%, Hr = 0.74 Method repeatability: RSD = 1.28%, Hr = 0.86 (expected content: 50% w/w, RSD criterion < 1.49%)
<b>Accuracy</b> <b>n = 5</b> (% Recovery)	1 <sup>st</sup> level (12.24%): 99.09% (RSD = 0.90%) (RSD criterion < 1.84%) 2 <sup>nd</sup> level (40.82%): 98.31% (RSD = 0.47%) (RSD criterion < 1.53%)
<b>Interference/ Specificity</b>	There are no interfering peaks (Injection of blank, folpet standard solution, sample solution, blank formulation solution, impurity CCl <sub>4</sub> , impurity PMM and fortified sample (with CCl <sub>4</sub> and PMM) solutions). Specific method.
<b>Comment</b>	-

### Conclusion

The analytical method for the determination of the active substance in the plant protection product SAP50SCF 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 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 perchloromethylmercaptan and carbon tetrachloride in FOLPEC was fully validated according to SANCO/3030/99 rev. 5.
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Reference:	KCP 5.1.1/02
Report	FOLPET 500 g/L SC (SAP50SCF): Physical, chemical and technical properties of the plant protection product, Morais, F., 2022, Report no EF/375/21 – Final Report – Annex 2.
Guideline(s):	Yes, SANCO/3030/99 rev. 5
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Reference:	KCP 5.1.1/03
Report	FOLPET 500 g/L SC (SAP50SCF): Physical, chemical and technical properties of the plant protection product, Morais, F., 2022, Report no EF/375/21 – Final Report – Annex 3.
Guideline(s):	Yes, SANCO/3030/99 rev. 5
Deviations:	No
GLP:	Yes
Acceptability:	Yes

- **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

<b>Injector method</b>	<i>Injection volume</i>	5.00 µL
	<i>Pre-inj dwell time</i>	3 s
	<i>Post-inj dwell time</i>	3 s
<b>GC method</b>	<i>Temperature</i>	70 °C for 5 minutes 15.0 °C/min until 220 °C (maintain for 5 minutes)
	<i>Column</i>	TG-5MS, 30 m × 0.25 mm × 0.25 µm film thickness
	<i>S/SL mode</i>	Split
	<i>Inlet temperature</i>	280 °C
	<i>Split flow</i>	7.5 mL/min (constant flow)
	<i>Carrier flow</i>	1.500 mL/min
<b>Detector Method (MS/MS)</b>	<i>Temperature</i>	280 °C
	<i>Ion source</i>	230 °C
	<i>Start time</i>	7.5 minutes
	<i>Ionization mode</i>	EI
	<i>Ion polarity</i>	Positive
	<i>Acquisition mode</i>	<u>SRM conditions</u> Scan #1 (SRM1) Precursor mass: 149 amu

		<ul style="list-style-type: none"> <li>Q3 Start mass: 78.995 amu</li> <li>Q3 End mass: 79.005 amu</li> <li>Scan time: 0.2 sec</li> <li>Collision energy: 20</li> </ul> <p>Scan #2 (SRM2)</p> <ul style="list-style-type: none"> <li>Precursor mass: 151 amu</li> <li>Q3 Start mass: 115.995 amu</li> <li>Q3 End mass: 116.005 amu</li> <li>Scan time: 0.2 sec</li> <li>Collision energy: 10</li> </ul>
<b>Retention times</b>	<i>PMM</i>	Around 8 minutes

### Standard solution preparation

Weigh about 50 mg  $\pm$  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 (%)
<b>L1</b>	0.15	10	0.00012	0.0813
<b>L2</b>	0.25	10	0.00020	0.1355
<b>L3</b>	0.35	10	0.00028	0.1897
<b>L4</b>	0.50	10	0.00040	0.2710
<b>L5</b>	0.70	10	0.00056	0.3794

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

### Sample solution preparation

Weigh approximately 123 mg  $\pm$  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  $\mu$ m disk filter. From this solution transfer 1.2 mL into a 10 mL volumetric flask and complete the volume with toluene (0.15 mg/mL). Prepare in duplicate.

- **Carbon tetrachloride (CCl<sub>4</sub>)**

## **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<sub>4</sub>) is quantified using a GLC method with MS/MS detection, operating in SRM (Single Reaction Monitoring) mode.

### Chromatographic conditions

<b>Injector method</b>	<i>Injection volume</i>	5.00 $\mu$ L
	<i>Pre-inj dwell time</i>	3 s
	<i>Post-inj dwell time</i>	3 s
<b>GC method</b>	<i>Temperature</i>	40 °C for 4 minutes 45.0 °C/min until 250 °C (maintain for 11 minutes)



	<i>Column</i>	TG-5MS, 30 m × 0.25 mm × 0.25 µm film thickness
	<i>S/SL mode</i>	Split
	<i>Inlet temperature</i>	200 °C
	<i>Split flow</i>	24.0 mL/min (constant flow)
	<i>Carrier flow</i>	1.200 mL/min
<b>Detector Method (MS/MS)</b>	<i>Temperature</i>	250 °C
	<i>Ion source</i>	250 °C
	<i>Start time</i>	3.2 minutes
	<i>Ionization mode</i>	EI
	<i>Ion polarity</i>	Positive
	<i>Acquisition mode</i>	<u>SRM conditions</u>
		Scan #1 (SRM1) <ul style="list-style-type: none"> <li>· Precursor mass: 82 amu</li> <li>· Q3 Start mass: 46.995 amu</li> <li>· Q3 End mass: 47.005 amu</li> <li>· Scan time: 0.2 sec</li> <li>· Collision energy: 20</li> </ul> Scan #2 (SRM2) <ul style="list-style-type: none"> <li>· Precursor mass: 117 amu</li> <li>· Q3 Start mass: 81.995 amu</li> <li>· Q3 End mass: 82.005 amu</li> <li>· Scan time: 0.2 sec</li> <li>· Collision energy: 10</li> </ul>
<b>Retention times</b>	<i>CCl<sub>4</sub></i>	Around 3.6 minutes

### Standard solution preparation

Weigh about 100 mg ± 10 mg of CCl<sub>4</sub> 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 <sup>1</sup> (%)
<b>L1</b>	0.10	10	0.00008	0.054
<b>L2</b>	0.15	10	0.00012	0.081
<b>L3</b>	0.25	10	0.00020	0.136
<b>L4</b>	0.40	10	0.00032	0.217
<b>L5</b>	0.70	10	0.00056	0.379

<sup>1</sup> For samples at 0.15 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 1.2 mL into a 10 mL volumetric flask and complete the volume with dichloromethane (0.15 mg/mL). Prepare in duplicate.

## Validation - Results and discussions

**Table 5.2-2: Methods suitable for the determination of the relevant impurities in plant protection product (PPP) SAP50SCF**

	<b>PMM max. 1.75 g/L</b>	<b>CCl<sub>4</sub> max. 2 g/L</b>
<b>Author(s), year</b>	Morais, F., 2022	Morais,F., 2022
<b>Principle of method</b>	GLC MS/MS	GLC MS/MS
<b>Linearity (linear between mg/L) (correlation coefficient, expressed as r)</b>	Range: [0.0865% - 0.4073%], [0.000128 – 0.000601 mg/mL] $y = 7265938764.3x - 474815.9$ $r = 0.9975$	Range: [0.0542% - 0.3798%], [0.000080 – 0.000561 mg/mL] $y = 34173269100.0x - 218413.5$ $r = 0.9992$
<b>Precision – Repeatability Mean n = 5 (%RSD)</b>	System rep.: RSD = 2.77%, Hr = 0.77 Method rep.: RSD = 3.17%, Hr = 0.88 (expected content: 0.14% w/w, RSD criterion < 3.60%)	System rep.: RSD = 1.54%, Hr = 0.39 Method rep.: RSD = 3.15%, Hr = 0.80 (expected content: 0.08% w/w, RSD criterion < 3.92%)
<b>Accuracy n = 5 (% Recovery)</b>	1 <sup>st</sup> level (0.14%): 93.39% (RSD = 2.04%) (RSD criterion < 3.60%) 2 <sup>nd</sup> level (0.29%): 91.15% (RSD = 1.85%) (RSD criterion < 3.23%)	1 <sup>st</sup> level (0.08%): 93.67% (RSD = 2.97%) (RSD criterion < 3.92%) 2 <sup>nd</sup> level (0.22%): 98.31% (RSD = 1.58%) (RSD criterion < 3.37%)
<b>Interference/ Specificity</b>	There are no interfering peaks (Injection of blank, PMM standard solution, sample solution, blank formulation solution, impurity CCl <sub>4</sub> and fortified sample (with CCl <sub>4</sub> ) solutions). Specific method.	There are no interfering peaks (Injection of blank, CCl <sub>4</sub> standard solution, sample solution, blank formulation solution, impurity PMM and fortified sample (with PMM) solutions). Specific method.
<b>LOQ</b>	LOQ=0.144% w/w	LOQ=0.081% w/w
<b>Comment</b>	Result: < LOQ (0.0144%)	Result: < LOQ (0.081%)

## Conclusion

The analytical methods for the determination of PMM and CCl<sub>4</sub> in the plant protection product SAP50SCF 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)

CIPAC Handbook 1B (page 1847), 75/WP/M for the quantification of folpet in wettable powders is applicable to Folpet 50 SC.

## 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 folpet for the generation of pre-authorization data is given in the following table. 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: sum of folpet and phthalimide expressed as folpet, phthalimide 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 phthalimide, expressed as folpet.  Processed commodities	Primary	Folpet: 0.01mg/kg Phthalimide: Grain&Whole Plant: 0.01 mg/kg Straw: 0.05 mg/kg  Folpet (each matrix): 0.01mg/kg Phthalimide (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  LC-QTRAP  LC-MS/MS	Jooß, S., 2022/ New study (KCP 5.1.2/01)  Gordo, J, 2022/ New study (KCP 5.1.2/10)  Jooß, S., 2022/ New study (KCP 5.1.2/02)
	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)
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	0.15 mg SAP50SCF/L	HPLC	██████████, 2011 / new study (KCP 5.1.2/03 equivalent to KCP 10.2.1/01)
		0.024 mg folpet/L	HPLC with UV detection	██████████, 2007 / new study (KCP 5.1.2/04 equivalent to KCP 10.2.1/02)
		0.041 mg folpet/L	HPLC with UV detection	Grade and Wydra, 2007 / new study (KCP 5.1.2/05 equivalent to KCP 10.2.1/03)

<b>Component of residue definition:</b> <b>sum of folpet and phthalimide expressed as folpet, phthalimide expressed as folpet and folpet</b>				
<b>Matrix type</b>	<b>Method type</b>	<b>Method LOQ</b>	<b>Principle of method (i.e. GC-MS or HPLC-UV)</b>	<b>Author(s), year / missing / EU agreed</b>
		0.024 mg folpet/L	HPLC with UV detection	Grade and Wydra, 2007 / new study (KCP 5.1.2/06 equivalent to KCP 10.2.1/04)
		0.00213 mg folpet/L	HPLC-MS/MS	Hemm, 2024 / new study (KCP 5.1.2/11 equivalent to KCP 10.2.1/05)
		150 mg folpet/L	HPLC	Turner, 2009 / new study (KCP 5.1.2/07 equivalent to KCP 10.6.2/01)
		150 mg folpet/L	HPLC	Turner, 2009 / new study (KCP 5.1.2/08 equivalent to KCP 10.6.2/02)
	Confirmatory (if required)	-	-	-
Dosage solution samples (Ecotoxicology)  Folpet	Primary	30.10 mg folpet/L	HPLC with UV detection	Schreitmüller, 2016 / new study (KCP 5.1.2/09)
	Confirmatory (if required)	-	-	-
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 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 folpet (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	Sum of folpet and phtalimide, expressed as folpet	0.03* mg/kg	<del>Reg. (EU) 2022/93</del> Reg. (EU) 2023/1042
Plant, high acid content		0.03* mg/kg	<del>Reg. (EU) 2022/93</del> Reg. (EU) 2023/1042
Plant, high protein/high starch content (dry commodities)		0.07* mg/kg	<del>Reg. (EU) 2022/93</del> Reg. (EU) 2023/1042
Plant, high oil content		0.07* mg/kg	<del>Reg. (EU) 2022/93</del> Reg. (EU) 2023/1042
Plant, difficult matrices (hops, spices, tea)		0.1* mg/kg	<del>Reg. (EU) 2022/93</del> Reg. (EU) 2023/1042
Muscle	Phthalimide, expressed as folpet	0.05* mg/kg	<del>Reg. (EU) 2022/93</del> Reg. (EU) 2023/1042
Milk		0.05* mg/kg	<del>Reg. (EU) 2022/93</del> Reg. (EU) 2023/1042
Eggs		0.05* mg/kg	<del>Reg. (EU) 2022/93</del> Reg. (EU) 2023/1042
Fat		0.05* mg/kg	<del>Reg. (EU) 2022/93</del> Reg. (EU) 2023/1042
Liver, kidney		0.05* mg/kg	<del>Reg. (EU) 2022/93</del> 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 <sup>3</sup>	AOEL sys/AOEL inhal: 0.1 mg/kg bw/d
Tissue (meat or liver)	Not applicable (EFSA, 2009)	Not required (EFSA, 2009)	Not classified as T / T+ (EFSA, 2009)
	No residue definition for body fluids/tissue is set (RAR, 2018)	0.01 mg/kg	General limit according to SANTE/2020/12830, Rev.2
Body fluids		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.

### 5.3.2.2 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 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 5.3-2: 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: Sum of folpet and phthalimide, 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 (dry)	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)
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 5.3-3: Statement on extraction efficiency**

	Method for products of plant origin
Required, available from:	A cross-validation study on plant matrices has been performed; please

	<b>Method for products of plant origin</b>
	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 SAP50SCF / Folpet 500 SC product is intended to be used in cereals (wheat and barley). Sufficient analytical methods for the determination of folpet (Sum of folpet and phthalimide, 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 <sup>14</sup>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.

### 5.3.2.3 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 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 5.3-4: 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

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
				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	LC-MS/MS	Schlewitz, P. (2015) / new study under EU review Report no R B4281
		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	0.02 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 5.3-5: 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.

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

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

**Table 5.3-6: 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.

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

An overview on the acceptable methods and possible data gaps for analysis of 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 5.3-7: 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) /

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
				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.

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

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

**Table 5.3-8: 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 <sup>3</sup>	GC-MS	Aris, D. (2012) / new study under EU review Report no ZEF0006
Confirmatory	According to SANTE/2020/12830 rev. 2 <i>if the analytical detection 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.</i> 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<sup>3</sup> is available.  
The detailed of analytical method is presented in Appendix 2.

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

Component of residue definition: <b>Not applicable</b> 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.

### 5.3.2.8 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	FOLPET 500 g/L SC (SAP50SCF): Physical, chemical and technical properties of the plant protection product Report No EF/375/21 – Final Report: Annex 1 – Folpet method validation and quantification ASCENZA Agro, S.A. GLP Unpublished	N	ASCENZA Agro, S.A.
KCP 5.1.1/02	Morais, F.	2022	FOLPET 500 g/L SC (SAP50SCF): Physical, chemical and technical properties of the plant protection product Report No EF/375/21 – Final Report: Annex 2 – PMM method validation and quantification ASCENZA Agro, S.A. GLP Unpublished	N	ASCENZA Agro, S.A.
KCP 5.1.1/03	Morais, F.	2022	FOLPET 500 g/L SC (SAP50SCF): Physical, chemical and technical properties of the plant protection product Report No EF/375/21 – Final Report: Annex 3 – CCl <sub>4</sub> method validation and quantification ASCENZA Agro, S.A. GLP Unpublished	N	ASCENZA Agro, S.A.
KCP 5.1.2/01	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 5.1.2/02	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 Northern Europe – 2021 Report No S22-04739 Eurofins Agroscience Services. GLP Unpublished	N	ASCENZA Agro, S.A.
KCP 5.1.2/03	██████	2011	Acute toxicity of Folpet Sapec 500 SC to Rainbow Trout ( <i>Oncorhynchus mykiss</i> ) in a 96-hour semi static test ██████ GLP Unpublished	Y	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 5.1.2/04	██████	2007	Acute toxicity of Folpet 80 WG to Rainbow Trout ( <i>Oncorhynchus mykiss</i> ) in a 96-hour semi static test ██████ GLP Unpublished	Y	ASCENZA Agro, S.A.
KCP 5.1.2/05	Grade, R., Wydra, V.	2007	Acute toxicity of Folpet 80 WG to <i>Daphnia magna</i> in a semi static 48-hour immobilization test Ibacon Report No. 33892220 GLP Unpublished	N	ASCENZA Agro, S.A.
KCP 5.1.2/06	Grade, R., Wydra, V.	2007	Influence of Folpet technical to <i>Daphnia magna</i> in a reproduction test Ibacon Report No. 33881221 GLP Unpublished	N	ASCENZA Agro, S.A.
KCP 5.1.2/07	Turner, B.	2009	Analysis of Folpet 80% WG Spray Solutions Huntingdon Life Sciences Report No. ACX0104 GLP Unpublished	N	ASCENZA Agro, S.A.
KCP 5.1.2/08	Turner, B.	2009	Analysis of Folpet 80% WG Spray Solution Huntingdon Life Sciences Report No. ACX0105 GLP Unpublished	N	ASCENZA Agro, S.A.
KCP 5.1.2/09	Schreitmüller, J.	2016	Analysis of Folpet in dosage solutions from Honey Bee Larvae Toxicity Study TRC14-245BA IES Report No. 20150171 GLP Unpublished	N	ASCENZA Agro, S.A.
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 5.1.2/11	Hemm, C.	2024	Analysis of folpet in Test Samples obtained from AscDaph study (CLOVER-A-01-2023) Eurofins Report No. S23-106026 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 5.2/01	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	Sapac 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	Sapac 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 Agrosience Services Chem SAS Report No.: S14-05779 Date: 24/03/2016 GLP: yes Unpublished	N	Sapac 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 Source: Eurofins Agrosience Services Chem GmbH Report No.: S16-00559 (BEL-1601V) Date: 24/03/2016 GLP: yes Unpublished	N	Sapac Agro S.A. and 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 Agrosience Services Chem GmbH Report No.: S16-00559 (BEL-1601V) Date: 29/04/2016 GLP: yes Unpublished	N	Sapac 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 5.2/06	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	Saptec 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	Saptec 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	Saptec Agro S.A. and ADAMA
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	Saptec 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	Saptec 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 5.2/11	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	Sapac 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	Sapac 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	Sapac Agro S.A. and ADAMA
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	Sapac 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	Sapac 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 <sup>14</sup> C-metabolism Studies for the Determination of Folpet and Metabolites in Wheat	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
			Report VAL 25/21 Laboratorio de Residuos de Pesticidas - ASCENZA AGRO, S.A. GLP Unpublished		

**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
-	-	-	-	-	-

**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
-	-	-	-	-	-

**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 folpet

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

##### A 2.1.1.1 Analytical method 1

Comments of zRMS:	<p>The method was evaluated by zRMS-Greece in RR of SAP50SCF (December 2023).</p> <p><u>zRMS-Greece comments:</u>  <i>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.</i></p> <p><u>zRMS-PL comments:</u>  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</p> <p>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.</p>
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Reference:	KCP 5.1.2/01
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

### 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 <sub>18</sub> , 4x3 mm, Art. No. AJO-8762			
Column	Supelco Ascentis Express C <sub>18</sub> (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 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]
	0.00	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 C <sub>18</sub> , 4x3 mm, Art. No. AJO-8762)			
Column	Supelco Ascentis Express C <sub>18</sub> (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 <sub>18</sub> , 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 <sub>18</sub> , 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 column oven 1290 series			
Pre-column	Phenomenex C <sub>18</sub> , 4 x 3 mm			
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 <sub>18</sub> (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 material)	0.01	82.0	87.0	Mass Transition <i>m/z</i> 313 =>130
		0.1	91.9		
	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 <i>m/z</i> 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 <i>m/z</i> 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 <i>m/z</i> 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 <i>m/z</i> 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 <i>m/z</i> 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 <i>m/z</i> 313 =>130
		0.1	90.4		
	Wheat (grain)	0.01	97.6	94.2	

Analyte	Matrix	Fortification level (mg/L) (n = 5)	Mean recovery (%)	RSD (%)	Comments
		0.1	90.7		Mass Transition <i>m/z</i> 315 =>130
	Wheat (straw)	0.01	107	107	
		0.1	107		
	Wheat (green material)	0.01	95.2	92.2	
		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)	<p>Correlation of peak area of different standard solutions with their corresponding concentrations, using a linear regression:</p> <p>Folpet grain(quantitation): R: 0.9995 Calibration curve: <math>y = 1.0 x + 0.00265</math> number of data points = 8</p> <p>Folpet grain(confirmation): R: 0.9988 Calibration curve: <math>y = 1 x + 0.00558</math> number of data points = 8</p> <p>Phthalimide grain/ green material(quantitation): R: 0.9999 Calibration curve: <math>y = 2.44 x - 0.00129</math> number of data points = 8</p> <p>Phthalimide grain/ green material(confirmation): R: 0.9996 Calibration curve: <math>y = 0.813 x + 0.00115</math> number of data points = 8</p> <p>Phthalic Acid (quantitation): R: 0.9992 Calibration curve: <math>y = 0.474 x - 0.00558</math> number of data points = 8</p> <p>Phthalic Acid (confirmation): R: 0.9997 Calibration curve: <math>y = 1.14x - 0.0258</math> number of data points = 8</p> <p>Phthalamic Acid grain (quantitation): R: 0.9999 Calibration curve: <math>y = 3.16e+003 x + 6.95e+003</math> number of data points = 8</p> <p>Phthalamic Acid grain (confirmation): R: 0.9998 Calibration curve: <math>y = 2.38e+004 x + 3.11e+004</math> number of data points = 8</p>

	Folpet
Calibration range	<p>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</p> <p>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)</p> <p>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</p> <p>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</p>
Assessment of matrix effects is presented	Yes
Limit of determination/quantification	<p>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)</p> <p>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)</p>
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.

## 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 2.1.1.2 Analytical method 2

Comments of zRMS:	<p>The method was evaluated by zRMS-Greece in RR of SAP50SCF (December 2023).</p> <p><u>zRMS-Greece comments:</u> 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.</p> <p><u>zRMS-PL comments:</u> Below are some errors that the evaluator corrected.</p>
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	<p>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.</p> <p>With regard to selectivity, accuracy and precision, the analytical methods were applied successfully for each analytical set when analysing the samples of the study.</p>
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Reference:	KCP 5.1.2/02
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):	<p>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.</p> <p>SANTE/2020/12830, rev.1 (Guidance Document on Pesticide Analytical Methods for Risk Assessment and Post-approval Control and Monitoring Purposes).</p> <p>ENV/JM/MONO(2007)17 (OECD Guidance Document on Pesticide Residue Analytical Methods).</p>
Deviations:	No
GLP:	Yes
Acceptability:	Fit for purpose

### 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 Malt Sprouts, Dried 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 <sub>18</sub> (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 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]
	0.00	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 column oven 1290 series			
Pre-column	Phenomenex C18, 4 x 3 mm			
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 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 $\Rightarrow$ 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 $\Rightarrow$ 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		
		4	0.01	102	7.9	

Analyte	Matrix	n=x	Fortification level (mg/L)	Mean recovery (%)	RSD (%)	Comments		
	Dried-Brewers Grain		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 $\Rightarrow$ 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 $\Rightarrow$ 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. (%)
<b>Folpet (Mass Transition <math>m/z</math> 315→130 (Quantification))</b>							
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. (%)
<b>Phthalimide (Mass Transition <math>m/z</math> 148→102 (Quantification))</b>							
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. (%)
<b>Phthalic Acid (Mass Transition <math>m/z</math> 165→77 (Quantification))</b>							
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. (%)
<b>Phthalamic Acid (Mass Transition m/z 166→130 (Quantification))</b>							
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**

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

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



	Folpet – Phthalimide - Phthalic Acid - Phthalamic Acid
	<p>Phthalamic Acid</p> <p>0.015 to 1.5 mg reference item/L</p> <p>1.875 ng/mL to 187.5 ng/mL for malt sprouts, corresponding to 0.015 mg/kg to 1.5 mg/kg</p> <p>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</p>
Assessment of matrix effects is presented	Yes
Limit of determination/quantification	<p>For folpet and phthalimide in all matrices:</p> <p>LOQ = 0.01 mg/kg</p> <p>LOD = 0.003 mg/kg</p> <p>For phthalic acid and phthalamic acid in all matrices:</p> <p>LOQ = 0.05 mg/kg</p> <p>LOD = 0.015 mg/kg</p>

## Conclusion

With regard to selectivity, accuracy and precision, the analytical methods were applied successfully for each analytical set when analysing the samples of the study. 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 2.1.1.3 Analytical method 3

Comments of zRMS:	<p>The method was evaluated by zRMS-Greece in RR of SAP50SCF (December 2023).</p> <p><u>zRMS-Greece comments:</u></p> <p><i>The analytical method is considered to be fit for purpose.</i></p> <p>LOQ = 0.15 mg test item/L</p>
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Reference:	KCP 5.1.2/03
Report	Acute toxicity of Folpet Sapec 500 SC to Rainbow Trout ( <i>Oncorhynchus mykiss</i> ) in a 96-hour semi static test, [REDACTED]
Guideline(s):	OECD No. 203 (1992)
Deviations:	No
GLP:	Yes
Acceptability:	Fit for purpose

## Materials and methods

The purpose of the analytical part of this study was to verify the concentration of the test item in the test medium.

Method for determination: HPLC-method

### HPLC-conditions

HPLC-System:	LaChrom, Merck Hitachi		
Column:	UltraSep ES RP 18 (250 x 4 mm)		
Oven temperature:	25 °C		
Detector:	UV-Vis-Detection		
Detection Wave Length:	210 nm		
Mobile Phase:	<p>A: acetonitrile containing 5 % pure water</p> <p>B: pure water</p>		
Gradient:	Time [min]	% A	% B

	0	65	35
	3	65	35
	4	90	10
	6	90	10
	6.1	65	35
	12	65	35
Flow Rate:	1 mL / min		
Injection Volume:	99 µL		
Integration Software:	EZChrom Elite		

## Results and discussions

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

Matrix	Analyte	Fortification level (mg/L) (n = 4)	Mean recovery (%)	RSD (%)	Comments
Test water	Folpet	0.15	96.25	Overall = 6 (n = 12)	Overall mean recovery: 94 % (n = 12)
		0.5	97.5		
		2.5	89.5		

**Table A 4: Characteristics for the analytical method used for validation of folpet residues in test water**

	Folpet
Specificity	HPLC-UV 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: R <sup>2</sup> : 0.9999 Calibration curve: $y = 1811038 * x - 2541$ number of data points = 8
Calibration range	0.01 to 0.75 mg reference item/L
Assessment of matrix effects is presented	Yes, chromatogram of control at 0 h presented
Limit of determination/quantification	LOD = 0.002 mg reference item/L LOQ = 0.15 mg test item/L

## Conclusion

The method used was not validated according to no specific guideline, but it is considered as reliable and fit for purpose as all the relevant validity criteria were assessed.

### A 2.1.1.4 Analytical method 4

Comments of zRMS:	<p>The method was evaluated by zRMS-Greece in RR of SAP50SCF (December 2023).  <u>zRMS-Greece comments:</u>  <i>The analytical method is considered to be fit for purpose.</i></p> <p><u>zRMS-PL comments:</u>  The missing Table A6 was added by the Evaluator.  LOQ = 0.03 mg test item/L</p>
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Reference: KCP 5.1.2/04

Report Acute toxicity of Folpet 80 WG to Rainbow Trout (*Oncorhynchus mykiss*) in a 96-hour semi static test, [REDACTED]

Guideline(s): OECD No. 203 (1992)

Deviations: No  
GLP: Yes  
Acceptability: Fit for purpose

## 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.

Method for determination: HPLC with UV detection

### HPLC-conditions

HPLC-System:	LaChrom, Merck Hitachi		
Column:	US ES RP18, 250 * 4 mm		
Oven temperature:	25 °C		
Detector:	UV-Vis-Detection		
Detection Wave Length:	210 nm		
Mobile Phase:	A: acetonitrile B: pure water		
Gradient:	Time [min]	% A	% B
	0	65	35
	3	65	35
	4	90	10
	6	90	10
	6.1	65	35
	12	65	35
Flow Rate:	1.0 mL / min		
Injection Volume:	100 µL		
Integration Software:	EZ Chrom Elite		

## Results and discussions

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

Matrix	Analyte	Fortification level (mg/L)	Mean recovery (%)	RSD (%)	Comments
Test water	Folpet	0.02 (n = 4)	129.75	Overall = 15 (n = 24)	Overall mean recovery: 102 % (n = 24)
		0.03 (n = 4)	98.00		
		0.1 (n = 8)	97.75		
		0.5 (n = 8)	94.75		

**Table A 6: Characteristics for the analytical method used for validation of folpet residues in test water**

	Folpet
Specificity	HPLC 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: R <sup>2</sup> at least 0.9998 Calibration curve: y = 1210149 * x - 4691 number of data points = 6
Calibration range	0.008 to 0.3 mg reference item/L
Assessment of matrix effects is presented	Yes, chromatogram of control at 0 h presented
Limit of determination/quantification	LOD = 0.0007 mg test item/L LOQ = 0.03 mg test item/L

## Conclusion

The method used was not validated according to no specific guideline, but it is considered as reliable and fit for purpose as all the relevant validity criteria were assessed.

### A 2.1.1.5 Analytical method 5

Comments of zRMS:	<p>The method was evaluated by zRMS-Greece in RR of SAP50SCF (December 2023).</p> <p><u>zRMS-Greece comments:</u> <i>The analytical method is considered to be fit for purpose.</i></p> <p><u>zRMS-PL comments:</u> LOQ = 0.05 mg test item/L</p>
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Reference:	KCP 5.1.2/05
Report	Acute toxicity of Folpet 80 WG to <i>Daphnia magna</i> in a semi static 48-hour immobilization test, Grade, R., Wydra, V., 2007, Report No. 33892220
Guideline(s):	OECD No. 203 (1992)
Deviations:	Yes, the mean recovery of the fortification level of 0.05 mg test item/L was 111% (n=6, RSD=18) and thus slightly higher than the required value (70 – 110 %). This was only a minor deviation and was considered not to influence the integrity of the study.
GLP:	Yes
Acceptability:	Fit for purpose

## Materials and methods

The purpose of the analytical part of this study was to verify the concentrations of this test item in the test medium.

Method for determination: HPLC with UV detection

### HPLC-conditions

HPLC-System:	LaChrom, Merck Hitachi		
Column:	US ES RP18, 250 * 4 mm		
Oven temperature:	25 °C		
Detector:	UV-Vis-Detection		
Detection Wave Length:	210 nm		
Mobile Phase:	A: acetonitrile B: pure water		
Gradient:	Time [min]	% A	% B
	0	65	35
	3	65	35
	4	90	10
	6	90	10
	6.1	65	35
	12	65	35
Flow Rate:	1.0 mL / min		
Injection Volume:	100 µL		
Integration Software:	EZ Chrom Elite		

## Results and discussions

**Table A 6: Recovery results from method validation of folpet using the analytical method**

Matrix	Analyte	Fortification level (mg/L) (n = 6)	Mean recovery (%)	RSD (%)	Comments
Test water	Folpet	0.05	110.8	Overall = 13 (n = 18)	Overall mean recovery: 105 % (n = 18)
		0.20	98.3		
		2.0	104.8		

**Table A 7: Characteristics for the analytical method used for validation of folpet residues in test water**

	Folpet
Specificity	HPLC-UV 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: R <sup>2</sup> : at least 0.9998 Calibration curve: $y = 1379362 * x - 3984$ number of data points = 8
Calibration range	0.01 to 1 mg reference item/L
Assessment of matrix effects is presented	Yes, chromatogram of control at 0 h presented
Limit of determination/quantification	LOD = 0.0028 mg a.s./L LOQ = 0.05 mg test item/L

## Conclusion

The method used was not validated according to no specific guideline, but it is considered as reliable and fit for purpose as all the relevant validity criteria were assessed.

### A 2.1.1.6 Analytical method 6

Comments of zRMS:	<p>The method was evaluated by zRMS-Greece in RR of SAP50SCF (December 2023).</p> <p><u>zRMS-Greece comments:</u></p> <p><i>The analytical method is considered to be fit for purpose.</i></p> <p><u>zRMS-PL comments:</u></p> <p>LOQ = 0.03 mg test item/L</p>
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Reference:	KCP 5.1.2/06
Report	Influence of Folpet technical to <i>Daphnia magna</i> in a reproduction test, Grade, R., Wydra, V., 2007, Report No. 33881221
Guideline(s):	OECD guideline 211, adopted September 1998
Deviations:	No
GLP:	Yes
Acceptability:	Fit for purpose

## Materials and methods

The purpose of the analytical part of this study was to verify the concentrations of the test item in the test medium.

Method for determination: HPLC with UV detection

#### HPLC-conditions

HPLC-System:	LaChrom, Merck Hitachi		
Column:	UltraSep ES RP 18, 250 * 4 mm		
Oven temperature:	25 °C		
Detector:	UV-Vis-Detection		
Detection Wave Length:	210 nm		
Mobile Phase:	A: acetonitrile B: pure water		
Gradient:	Time [min]	% A	% B
	0	65	35
	3	65	35
	4	90	10
	6	90	10
	6.1	65	35
	12	65	35
Flow Rate:	1.0 mL / min		
Injection Volume:	100 µL		
Integration Software:	EZChrom Elite		

## Results and discussions

**Table A 8: Recovery results from method validation of folpet using the analytical method**

Matrix	Analyte	Fortification level (mg/L) (n = 12)	Mean recovery (%)	RSD (%)	Comments
Test water	Folpet	0.03	106.0	Overall = 9 (n = 33)	Overall mean recovery: 98% (n = 33)
		0.5	96.20		
		3.5	94.25		

**Table A 9: Characteristics for the analytical method used for validation of folpet residues in test water**

	Folpet
Specificity	HPLC-UV 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: R <sup>2</sup> : at least 0.9997 Calibration curve Low calibration range: $y = 1480268 * x - 2057$ High calibration range: $y = 1354069 * x - 21982$ number of data points = 5/calibration range
Calibration range	Due to the high concentration range of the measured samples the calibration range was split in a low and a high calibration range: Low calibration range: 0.01 to 1 mg test item/L High calibration range: 0.1 to 2 mg test item/L
Assessment of matrix effects is presented	Yes, chromatogram of control at 0 h presented
Limit of determination/quantification	LOD = 0.004 mg test item/L LOQ = 0.03 mg test item/L

## Conclusion

The method used was not validated according to no specific guideline, but it is considered as reliable and fit for purpose as all the relevant validity criteria were assessed.

### A 2.1.1.7 Analytical method 7

Comments of zRMS:	The method was evaluated by zRMS-Greece in RR of SAP50SCF (December 2023). <u>zRMS-Greece comments:</u> <i>The analytical method is considered to be fit for purpose.</i>
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	However, the number of recoveries per fortification level is not adequate.
	<u>zRMS-PL comments:</u>
	No LOQ is given.
	The method used was not validated according to current guidelines.

Reference:	KCP 5.1.2/07
Report	Analysis of Folpet 80% WG Spray Solutions, Turner, B., 2009, Report No. ACX0104
Guideline(s):	OECD Guideline 208
Deviations:	No
GLP:	Yes
Acceptability:	Fit for purpose

### Materials and methods

The content of the active substance, folpet, in the spray solutions was determined using a high performance liquid chromatography (HPLC) method based on conditions supplied by the Sponsor.

#### HPLC-conditions

Instrument:	Agilent 1200 Liquid Chromatograph
Column:	Nucleosil 120-5 C18, 5Hm (25 cm x 4.6 mm internal diameter)
Column temperature:	30 °C
Mobile phase composition:	Acetonitrile:0.1% v/v trifluoroacetic acid (aq) (45:55 v/v)
Flow Rate:	1.5 mL / min
Injection Volume:	5 µL
Detector:	UV at 254 nm
Retention times:	Approximately 3 minutes
Analysis time:	8 minutes

### Results and discussions

**Table A 10:** Characteristics for the analytical method used for validation of folpet residues in test water

	Folpet
Specificity	HPLC-UV blank value < 30 % LOQ
Calibration (type, number of data points)	R <sup>2</sup> : 0.9999 Calibration curve: $y = 1.243x - 5.259$ number of data points = 6
Calibration range	152.3 to 1523 mg/L
Assessment of matrix effects is presented	Yes
Limit of determination/quantification	<del>LOQ = 150 mg/L</del> No LOQ is given

### Conclusion

The method used was not validated according to no specific guideline, but it is considered as reliable and fit for purpose as all the relevant validity criteria were assessed.

#### A 2.1.1.8 Analytical method 8

Comments of zRMS:	The method was evaluated by zRMS-Greece in RR of SAP50SCF (December 2023). <u>zRMS-Greece comments:</u> The analytical method is considered to be fit for purpose. However, the number of recoveries per fortification level is not adequate.
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	<b>zRMS-PL comments:</b> No LOQ is given. The method used was not validated according to current guidelines.
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Reference:	KCP 5.1.2/08
Report	Analysis of Folpet 80% WG Spray Solutions, Turner, B., 2009, Report No. ACX0105
Guideline(s):	OECD Guideline 227
Deviations:	No
GLP:	Yes
Acceptability:	Fit for purpose

## Materials and methods

The content of the active substance, folpet, in the spray solutions was determined using a high performance liquid chromatography (HPLC) method based on conditions supplied by the Sponsor.

### HPLC-conditions

Instrument:	Agilent 1200 Liquid Chromatograph
Column:	Nucleosil 120-5 C18, 5Hm (25 cm x 4.6 mm internal diameter)
Column temperature:	30 °C
Mobile phase composition:	Acetonitrile:0.1% v/v trifluoroacetic acid (aq) (45:55 v/v)
Flow Rate:	1.5 mL / min
Injection Volume:	5 µL
Detector:	UV at 254 nm
Retention times:	Approximately 3 minutes
Analysis time:	8 minutes

## Results and discussions

**Table A 11:** Characteristics for the analytical method used for validation of folpet residues in test water

	Folpet
Specificity	HPLC-UV blank value < 30 % LOQ
Calibration (type, number of data points)	R <sup>2</sup> : 0.9999 Calibration curve: $y = 1.243x - 5.259$ number of data points = 6
Calibration range	152.3 to 1523 mg/L
Assessment of matrix effects is presented	Yes
Limit of determination/quantification	<del>LOQ = 150 mg/L</del> No LOQ is given

## Conclusion

The method used was not validated according to no specific guideline, but it is considered as reliable and fit for purpose as all the relevant validity criteria were assessed.

### A 2.1.1.9 Analytical method 9

Comments of zRMS:	The method was evaluated by zRMS-Greece in RR of SAP50SCF (December 2023). <b>zRMS-Greece comments:</b> <i>The analytical method is considered to be fit for purpose.</i> <i>However, the number of recoveries per fortification level is not adequate.</i>
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Reference:	KCP 5.1.2/09
Report	Analysis of Folpet in dosage solutions from Honey Bee Larvae Toxicity Study TRC14-245BA, Schreitmüller, J., 2016, Report No. 20150171
Guideline(s):	Not applicable.
Deviations:	No
GLP:	Yes
Acceptability:	Fit for purpose

## Materials and methods

The purpose of this study was the determination of the concentrations of Folpet in dose solutions from honeybee larvae toxicity study TRC14-245BA. In case of low levels of Folpet, the metabolite phthalimide should be analysed as well.

Method for determination: 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 Å; 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 discussions

**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 water**

	Folpet
Specificity	HPLC-UV blank value < 30 % LOQ
Calibration (type, number of data points)	R <sup>2</sup> : 0.9991 Calibration curve: y = 1x + 1 number of data points = 12
Calibration range	30.10 – 300.9 mg Folpet/L
Assessment of matrix effects is presented	Yes

	Folpet
Specificity	HPLC-UV blank value < 30 % LOQ
Limit of determination/quantification	LOQ = 30.10 mg folpet/L

## Conclusion

The method used was not validated according to no specific guideline, but it is considered as reliable and fit for purpose as all the relevant validity criteria were assessed.

### A 2.1.1.10 Analytical method 10

Comments of zRMS:	<p>The method was evaluated by zRMS-Greece in RR of SAP50SCF (December 2023).</p> <p><u>zRMS-Greece comments:</u>  <i>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.</i></p> <p><u>zRMS-PL comments:</u>  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.</p>
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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):	<p>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.</p> <p>SANTE/2020/12830, Rev.1: Guidance Document on Pesticide Analytical Methods for Risk Assessment.</p> <p>and Post-approval Control and Monitoring Purposes, 24/02/2021;</p> <p>SANTE/12682/2019, Guidance document on analytical quality control and method validation procedures for pesticide residues analysis in food and feed, 01/01/2020.</p> <p>OECD Series on Testing and Assessment, Number 9.</p>
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  $\approx$  1 minute. After this, 10 g of sodium sulfate anhydrous was added and shaken vigorously for some seconds, followed by other shaking step during  $\approx$  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 Na<sub>2</sub>SO<sub>4</sub> and shaken. The mixture was centrifuged for  $\approx$

5 minutes at  $\approx 3000$  rpm. The supernatant was then filtered through appropriate filters (PTFE,  $0.20\ \mu\text{m}$ ). 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  $\approx 2$  minutes on a mechanical shaker. Then, 0.8 mL of acidified water was added followed by another shaking step during  $\approx 5$  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  $\approx 11$  minutes on a mechanical shaker (Multi Reax). The mixture was centrifuged for  $\approx 5$  minutes at  $\approx 4000$  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  $\approx 11$  minutes on a mechanical shaker (Multi Reax). The mixture was centrifuged for  $\approx 5$  minutes at  $\approx 4000$  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 $1.8\ \mu\text{m}$ from Waters, $2.1 \times 100\ \text{mm}$		
Oven temperature:	40 °C		
Mobile Phase:	A: H <sub>2</sub> O:MeOH:1 M ammonium formate:formic acid (940:50:9:1, v/v) B: H <sub>2</sub> O: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 $\mu\text{L}$		
Autosampler temperature:	15 °C		
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  $\pm 0.1$  min)

Typical MRM Transition Ratio (MRM1/MRM2): 3.0 (with tolerance of  $\pm 30\%$ )

Typical MRM Transition Ratio (MRM1/MRM3): 1.6 (with tolerance of  $\pm 30\%$ )

##### 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  $\pm 0.1$  min)

#### LC-QTRAP-conditions for phthalic acid

LC-QTRAP System:	SCIEX Exion LC		
Column:	ACQUITY UPLC HSS T3 $1.8\ \mu\text{m}$ from Waters, $2.1 \times 100\ \text{mm}$		
Oven temperature:	40 °C		

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 °C		
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
	<p>Folpet: R: 0.99380 Calibration curve: <math>y = 2.21008x + 642.78720</math> number of data points = 7</p> <p>Phthalimide: R: 0.99912 Calibration curve: <math>y = 5.33986x + 430.98319</math> number of data points = 7</p>
Calibration range	<p>Folpet 0.0015 ng/μL to 0.0375 ng/μL, corresponding to 0.003 - 0.075 mg/kg</p> <p>Phthalimide 0.0015 ng/μL to 0.0375 ng/μL, corresponding to 0.003 - 0.075 mg/kg (MRM transition 146.0&gt;42.0) 0.0015 ng/μL to 0.050 ng/μL, corresponding to 0.003 - 0.1 mg/kg (MRM transition 146.0&gt;42.0)</p>
Assessment of matrix effects is presented	Yes
Limit of determination/quantification	<p>LOQ = 0.01 mg/kg LOD = 0.003 mg/kg</p>

## 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 2.1.1.11 Analytical method 11

Comments of zRMS:	<p>The analytical method for the determination of folpet in ISO test water was successfully validated within this study with regard to recovery, linearity of detector response, repeatability, specificity, matrix effect, stability of working solutions/ sample extracts, limit of quantification and limit of detection. The analytical methods fulfil the requirements of guideline SANTE/2020/12830 rev. 2/ 14/02/2023.</p> <p>LOQ = 0.00213 mg/L</p> <p>The method is acceptable.</p>
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Reference:	KCP 5.1.2/11
Report	Analysis of folpet in Test Samples obtained from AscDaph study (CLOVER-A-01-2023), Hemm, C., 2024, Report No. S23-106026
Guideline(s):	SANTE/2020/12830, rev. 2
Deviations:	No
GLP:	Yes
Acceptability:	Yes

## Materials and methods

To perform dose verification in samples obtained from an AscDaph study (CLOVER-A-01-2023) via analysis of folpet. Analysis was performed in accordance to guidance document SANTE/2020/12830, rev.2 for risk assessment.

Method Summary: Samples arrived at the test site on dry ice and were stored at  $\leq -18$  °C. On the day of work up the samples were allowed to thaw. The full sample container was weighted and the weight was recorded. 10 mL of Acetonitrile was used to transfer the sample to a glass vial in several steps (dilution

step 1 (f1)). Afterwards samples were shaken on a flatbed shaker for about 15 min at 300 rpm. The sample was further diluted with matrix blank solution (dilution step 2 (f2)). to be within the calibration range and measured with LC-MS/MS. After drying the empty sample container weight was recorded.

#### Preparation of Standard solutions

Stock solutions of the analyte were prepared by dissolving a weight of the test / reference items in Acetonitrile + 0.1 % formic acid . Solutions for fortification and calibration were obtained by (serial) dilution of the stock solutions.

Matrix-matched calibration solutions were prepared using final sample extracts of control (unreated) samples of a respective matrix which were then fortified with solvent standard solutions. All solutions were stored at typically 1 °C to 10 °C in a glass vial in the dark.

Chromatographic conditions for folpet in ISO test water				
HPLC system	Shimadzu HPLC system			
Column	Phenomenex Synergi Fusion-RP 80A, 50 mm x 2 mm, 4 µm, (Part No. 00B-4424-B0)			
Pre-column	HPLC guard column (KJ0-4282, Phenomenex) with 4 mm Fusion RP cartridge (AJ0-7556, Phenomenex)			
Column oven temperature	30 °C			
Injection volume	20 µL			
Mobile phases	Eluent A: Water + 10 mM Ammonium fomite + 0.1% formic acid Eluent B: Methanol			
Gradient	Time [min]	% Eluent A	% Eluent B	Flow [µL/min]
	0.1	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	0.1 min Waste 1.5 min MS/MS 3.0 min Waste			
Retention time	Folpet: approx. 2.2 min			

Mass spectrometric conditions						
MS system	SCIEX API 5500					
Ionisation type	Electrospray ionization (ESI)					
Polarity	Positive					
Scan type	MS/MS, Multiple Reaction Monitoring (MRM)					
Capillary voltage (IS)	5500 V	Ion spray 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	Mass transition monitored ( <i>m/z</i> )	Declustering potential (DP) [V]	Entrance potential (EP) [V]	Collision energy (CE) [V]	Cell exit potential (CXP) [V]	Dwell time [ms]
Folpet	315 ->130 <sup>#</sup>	11	10	37	10	200

	313 -> 130*	11	10	37	10	200
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# proposed (validation) and used (residue analysis, storage) for quantification. Both of the mass transitions listed can be used for quantification.

\* Used for quantification of Standard Stability

## Results and discussions

**Table A 16: 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	0.00213	107	12.8	Overall mean recovery: 10 %, overall mean RSD: 9.6% (n = 10)
		1.28	104	3.68	
		0.00213	102	17.9	Overall mean recovery: 103 %, overall mean RSD: 12.5 % (n = 10)
		1.28	104	4.45	

**Table A 17: Characteristics for the analytical method used for validation of folpet residues in test water**

	Folpet
Specificity	No significant interferences at the retention time of analyte in any of the blank matrix tested (< LOD).  - Quantifier mass transition $m/z$ 315 ->130 (evaluated and used for quantification) Qualifier mass transition $m/z$ 313 -> 130 (used for quantification of Standard Stability)
Calibration (type, number of data points)	Correlation of peak area of different standard solutions with their corresponding concentrations, using a linear regression: $r = 0.9999$ Calibration curve: $y = 3.88e+003 * x - 254$ number of data points = 7
Calibration range	0.300 – 30.0 ng/mL folpet with at least five (5) data points (corresponding to 0.0006 – 0.06 mg folpet /L,)
Assessment of matrix effects is presented	Yes. Deemed to be insignificant for ISO-water. Nevertheless, matrix-matched standards were used.
Limit of determination/quantification	LOD = 0.0006 mg folpet/L LOQ = 0.00213 mg folpet/L

## Conclusion

The method was successfully validated for determination of the analyte in ISO test water with an LOQ of 0.00213 mg/L and up to 1.28 mg/L (Folpet) according to guidance document(s) SANTE/2020/12830, rev. 2, for risk assessment with regard to selectivity, accuracy and precision, the analytical method was applied successfully for each analytical set when analysing the samples of the study.

### 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)

##### A 2.1.2.1.1 Analytical method 1

Comments of zRMS:	<p>The method was evaluated by zRMS-Greece in RR of SAP50SCF (December 2023).</p> <p><u>zRMS-Greece comments:</u></p> <p><i>The study is considered acceptable.</i></p> <p><i>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.</i></p>
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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  $\rightarrow$  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 (>  $\pm$  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 1: 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
		0.10	103.6	6.0	BEH C18 column $m/z$ 146.0 $\rightarrow$ 41.9
		0.01	104.7	5.4	Confirmation

Matrix	Analyte	Fortification level (mg/kg) (n = 5)	Mean recovery (%)	RSD (%)	Comments
	Phthalimide				ZORBAX SB-C3 column $m/z$ 146.0 → 41.9
		0.01	90.5	1.6	Quantification
		0.10	96.8	6.9	BEH C18 column $m/z$ 146.0 → 41.9
		0.01	95.6	3.5	Confirmation ZORBAX SB-C3 column $m/z$ 146.0 → 41.9
Tomato	Folpet	0.01	107.8	2.0	Quantification
		0.10	106.9	4.4	BEH C18 column $m/z$ 146.0 → 41.9
		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
		0.10	97.2	2.8	BEH C18 column $m/z$ 146.0 → 41.9
		0.01	105.1	4.0	Confirmation ZORBAX SB-C3 column $m/z$ 146.0 → 41.9

**Table A 2: 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)	<u>Grapes:</u> Primary method: $C=2.2834E-03 \times S + 1.32$ ( $r=0.99760$ ) Confirmatory method: $C=2.7252E-03 \times S + 0.99$ ( $r=0.99907$ )  <u>Tomato:</u> Primary method: $C=3.1875E-03 \times S + 1.15$ ( $r=0.99914$ ) Confirmatory method: $C=2.6706E-03 \times S + 0.97$ ( $r=0.99955$ )  8 data points	<u>Grapes:</u> Primary method: $C=5.3098E-04 \times S - 4.43$ ( $r=0.99996$ ) Confirmatory method: $C=5.5002E-04 \times S - 1.94$ ( $r=0.99988$ )  <u>Tomato:</u> Primary method: $C=5.3363E-04 \times S + 0.15$ ( $r=0.99994$ ) Confirmatory method: $C=5.1609E-04 \times S + 0.59$ ( $r=0.99979$ )  8 data points

	<b>Folpet</b>	<b>Phthalimide</b>
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

## 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 2.1.2.1.1.1 Independent laboratory validation

Comments of zRMS:	The method was evaluated by zRMS-Greece in RR of SAP50SCF (December 2023).  <u>zRMS-Greece comments:</u> <i>The study is considered acceptable.</i>
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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 3: 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	<i>Quantification:</i> BEH C18 column $m/z$ 146.0 → 41.9
		0.10	107	5	
		0.01	106	11	<i>Confirmation:</i> ZORBAX SB-C3 column; $m/z$ 146.0 → 41.9
		0.10	104	5	
	Phthalimide	0.01	93	8	<i>Quantification:</i> BEH C18 column $m/z$ 146.0 → 41.9
		0.10	93	6	
		0.01	91	8	<i>Confirmation:</i> ZORBAX SB-C3 column; $m/z$ 146.0 → 41.9
		0.10	98	8	
Tomato	Folpet	0.01	107	5	<i>Quantification:</i> BEH C18 column $m/z$ 146.0 → 41.9
		0.10	108	4	
		0.01	90	8	<i>Confirmation:</i> ZORBAX SB-C3 column; $m/z$ 146.0 → 41.9
		0.10	93	8	
	Phthalimide	0.01	74	6	<i>Quantification:</i> BEH C18 column $m/z$ 146.0 → 41.9
		0.10	87	1	
		0.01	77	7	<i>Confirmation:</i> ZORBAX SB-C3 column; $m/z$ 146.0 → 41.9
		0.10	95	4	

**Table A 4: 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:	LC-MS/MS Primary method: $m/z$ 146.0 → 41.9 (Column: BEH C18) Confirmatory method:

	$m/z$ 146.0 $\rightarrow$ 41.9 (Column: ZORBAX SB-C3) blank value < 30 % LOQ	$m/z$ 146.0 $\rightarrow$ 41.9 (Column: ZORBAX SB-C3) blank value < 30 % LOQ
Calibration (type, number of data points)	<u>Grapes:</u> Primary method: $y=105x-222$ ( $r^2=0.9946$ ) 8 data points  Confirmatory method: $y=20976x-27701$ ( $r^2=0.9958$ ) 8 data points  <u>Tomato:</u> Primary method: $y=161x-69$ ( $r^2=0.9920$ ) 7 data points  Confirmatory method: $y=19380x-18631$ ( $r^2=0.9992$ ) 8 data points	<u>Grapes:</u> Primary method: $y=245x-279$ ( $r^2=0.9944$ ) 7 data points  Confirmatory method: $y=40519x+79606$ ( $r^2=0.9994$ ) 8 data points  <u>Tomato:</u> 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	<u>Grapes and tomato:</u> LOQ = 0.01 mg/kg LOD = 0.003 mg/kg	<u>Grapes and tomato:</u> 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 2.1.2.1.2 Analytical method 2

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

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 5: 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	
	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

Matrix	Analyte	Fortification level (mg/kg) (n = 5)	Mean recovery (%)	RSD (%)	Comments
		0.10	106	2.7	m/z 148 → 130
		0.01	89	19	Confirmation
		0.10	110	2.4	m/z 148 → 102

**Table A 6: 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)	<u>Barley grain:</u> Primary method: y=1870.7952x + 1485.1669 (r=0.9997) Confirmatory method: y=3099.4006x + 2092.5616 (r=0.9999)  6 data points  <u>Sunflower seeds:</u> Primary method: y=3772.8481x + 2601.5185 (r=0.9998) Confirmatory method: y=6706.3128x + 5697.6074 (r=0.9998)  7 data points	<u>Barley grain:</u> Primary method: y=22397.6047x – 1266.5626 (r=0.9996) Confirmatory method: y=14229.7766x – 3666.1278 (r=0.9995)  6 data points  <u>Sunflower seeds:</u> 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 2.1.2.1.2.1 Independent laboratory validation

Comments of zRMS:	The method was evaluated by zRMS-Greece in RR of SAP50SCF (December 2023).  <u>zRMS-Greece comments:</u> <i>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%.</i>
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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 ( $\geq 20\%$ ). 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 7: 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 $\rightarrow$ 260
		0.1	77	11	
		0.01	81	14	Confirmation $m/z$ 298 $\rightarrow$ 130
		0.1	78	11	
	Phthalimide	0.01	89	26	Quantification $m/z$ 148 $\rightarrow$ 130
		0.1	101	16	
		0.01	82	27	Confirmation $m/z$ 148 $\rightarrow$ 102
		0.1	106	19	
Wheat grain	Folpet	0.01	60	7	Quantification $m/z$ 298 $\rightarrow$ 260
		0.1	71	7	
		0.01	62	11	Confirmation $m/z$ 298 $\rightarrow$ 130
		0.1	70	7	
	Phthalimide	0.01	79	17	

Matrix	Analyte	Fortification level (mg/kg) (n = 5)	Mean recovery (%)	RSD (%)	Comments
		0.1	93	6	Quantification $m/z$ 148 → 130
		0.01	79	6	Confirmation $m/z$ 148 → 102
		0.1	91	9	

**Table A 8: 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)	<u>Sunflower seeds:</u> 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  <u>Wheat grain:</u> 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	<u>Sunflower seeds:</u> 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  <u>Wheat grain:</u> 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 2.1.2.1.2.2 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 $^{14}\text{C}$ -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
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	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.
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Reference:	KCP 5.2/16
Report	Cross validation of an internal extraction method from LabRP vs. an Extraction Method Applied in <sup>14</sup> C-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 <sup>14</sup>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 <sup>14</sup>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.

### Extraction <sup>14</sup>C-Metabolism method

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  $\approx 1$  minute. After this, 10 g of sodium sulphate anhydrous was added and shaken vigorously for some seconds, follow by other shaking step during  $\approx 11$  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 Na<sub>2</sub>SO<sub>4</sub> and shaken. The mixture was centrifuged for  $\approx 5$  minutes at  $\approx 3000$  rpm. The supernatant was then filtered through appropriate filters (PTFE, 0.20  $\mu$ m). 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  $\approx 2$  minutes on a mechanical shaker. Then, 0.8 mL of acidified water was added followed by another shaking step during  $\approx 5$  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  $\approx 11$  minutes on a mechanical shaker (Multi Reax). The mixture was centrifuged for  $\approx 5$  minutes at  $\approx 4000$  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  $\approx 11$  minutes on a mechanical shaker (Multi Reax). The mixture was centrifuged for  $\approx 5$  minutes at  $\approx 4000$  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 2.1.2.2 Description of analytical methods for the determination of residues in animal matrices (KCP 5.2)

#### A 2.1.2.2.1 Analytical method 1

Comments of zRMS:	<p>The method was evaluated by zRMS-Greece in RR of SAP50SCF (December 2023).</p> <p><u>zRMS-Greece comments:</u></p> <p><i>The method is considered acceptable.</i></p> <p><i>However, 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.</i></p> <p><i>Therefore, the method is not appropriate for the determination of this analyte in milk.</i></p>
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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 ( $> \pm 20\%$ ). Matrix matched standards were used for quantification for all matrices, by default.

LOQ: 0.01 mg/kg in all matrices.

**Table A 9: 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	<i>Quantification</i> Column: BEH C18 $m/z$ 146.0 $\rightarrow$ 41.9
		0.1	103.7	3.2	
		0.01	107.8	0.9	<i>Confirmation</i> Column: ZORBAX SB-C3 $m/z$ 146.0 $\rightarrow$ 41.9
Meat	Phthalimide	0.01	104.4	3.9	<i>Quantification</i> Column: BEH C18 $m/z$ 146.0 $\rightarrow$ 41.9
		0.10	96.9	3.8	
		0.01	101.1	9.8	<i>Confirmation</i> Column: ZORBAX SB-C3 $m/z$ 146.0 $\rightarrow$ 41.9
Fat	Phthalimide	0.01	114.3	3.6	<i>Quantification</i> Column: BEH C18 $m/z$ 146.0 $\rightarrow$ 41.9
		0.10	108.1	3.1	
		0.01	104.6	2.6	<i>Confirmation</i> Column: ZORBAX SB-C3 $m/z$ 146.0 $\rightarrow$ 41.9
Liver	Phthalimide	0.01	82.3	5.7	<i>Quantification</i> Column: BEH C18 $m/z$ 146.0 $\rightarrow$ 41.9
		0.10	85.4	3.3	
		0.01	84.7	4.1	<i>Confirmation</i> Column: ZORBAX SB-C3 $m/z$ 146.0 $\rightarrow$ 41.9

**Table A 10: Characteristics for the analytical method used for validation of folpet residues in animal matrices**

	Phthalimide	
Specificity	Primary method	Confirmatory method
	LC – MS/MS Column: BEH C18 $m/z$ 146.0 $\rightarrow$ 41.9 blank value < 30 % LOQ	LC – MS/MS Column: ZORBAX SB-C3 $m/z$ 146.0 $\rightarrow$ 41.9 blank value < 30 % LOQ
Calibration (type, number of data points)	<u>Eggs:</u> $C=8.3630E-04xS-0.30$ (r=0.99952)  <u>Meat:</u> $C=7.1434E-04xS-0.72$ (r=0.99996)  <u>Fat:</u> $C=7.7537E-04xS+0.16$ (r=0.99978)  <u>Liver:</u> $C=8.6096E-04xS-0.25$ (r=0.99979)  8 data points	<u>Eggs:</u> $C=8.0830E-04xS+1.66$ (r=0.99974)  <u>Meat:</u> $C=5.7436E-04xS-0.31$ (r=0.99909)  <u>Fat:</u> $C=6.5612E-04xS+0.42$ (r=0.99870)  <u>Liver:</u> $C=6.5978E-04xS+0.83$ (r=0.99927)  8 data points

	Phthalimide
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
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 2.1.2.2.1.1 Independent laboratory validation

Comments of zRMS:	<p>The method was evaluated by zRMS-Greece in RR of SAP50SCF (December 2023).</p> <p><u>zRMS-Greece comments:</u> <i>The method is considered acceptable.</i></p> <p><u>zRMS-PL remark:</u> The method is not appropriate for the determination of phthalimide in milk.</p>
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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 11: 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	<i>Quantification</i> Column: BEH C18 <i>m/z</i> 146.0 → 41.9
		0.1*	82	8	
		0.01	90	4	<i>Confirmation</i> Column: ZORBAX SB-C3 <i>m/z</i> 146.0 → 41.9
		0.1	83	6	
Muscle	Phthalimide	0.01	86	3	<i>Quantification</i> Column: BEH C18 <i>m/z</i> 146.0 → 41.9
		0.1	88	4	
		0.01	87	3	<i>Confirmation</i> Column: ZORBAX SB-C3 <i>m/z</i> 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 12: Characteristics for the analytical method used for independent laboratory validation of folpet residues in animal matrices**

	Phthalimide	
Specificity	Primary method	Confirmatory method
	LC-MS/MS <i>m/z</i> 146.0 → 41.9 (Column: BEH C18) blank value < 30 % LOQ	LC-MS/MS <i>m/z</i> 146.0 → 41.9 (Column: ZORBAX SB-C3) blank value < 30 % LOQ
Calibration (type, number of data points)	<u>Muscle:</u> $y=895x+149$ ( $r^2=0.9948$ )  7 data points  <u>Liver:</u> $y=506x-670$ ( $r^2=0.9992$ )  8 data points	<u>Muscle:</u> $y=1720x+37$ ( $r^2=0.9986$ )  8 data points  <u>Liver:</u> $y=1419x+1268$ ( $r^2=0.9994$ )  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 2.1.2.2.2 Analytical method 2

Comments of zRMS:	The method was evaluated by zRMS-Greece in RR of SAP50SCF (December 2023).
	<u>zRMS-Greece comments:</u> <i>The method is considered acceptable.</i>

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  $\rightarrow$  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 (>  $\pm$  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 13: 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 $\rightarrow$ 130
		0.10	98	2.4	
		0.01	99	6.2	Confirmation $m/z$ 148 $\rightarrow$ 102
		0.10	99	2.1	
Egg	Phthalimide	0.01	98	4.5	Quantification $m/z$ 148 $\rightarrow$ 130
		0.1	91	4.2	
		0.01	96	3.1	Confirmation $m/z$ 148 $\rightarrow$ 102
		0.1	90	4.4	
Fat	Phthalimide	0.01	105	3.9	Quantification $m/z$ 148 $\rightarrow$ 130
		0.10	85	12	
		0.01	107	4.1	Confirmation $m/z$ 148 $\rightarrow$ 102
		0.10	85	11	

**Table A 14: Characteristics for the analytical method used for validation of folpet residues in animal matrices**

	Phthalimide	
Specificity	Primary method	Confirmatory method
	LC – MS/MS $m/z$ 148 → 130 blank value < 30 % LOQ	LC – MS/MS $m/z$ 148 → 102 blank value < 30 % LOQ
Calibration (type, number of data points)	<u>Milk:</u> $y=30383.6164x+71732.0083$ ( $r=0.9994$ ) <u>Fat:</u> $y=38568.9467x-42230.4916$ ( $r=0.9997$ ) <u>Egg:</u> $y=39609.6689x+13366.8278$ ( $r=0.9999$ ) 7 data points	<u>Milk:</u> $y=19134.7160x+41571.3771$ ( $r=0.9994$ ) <u>Fat:</u> $y=24129.5851x-36288.5510$ ( $r=0.9996$ ) <u>Egg:</u> $y=24884.0487x+17651.1391$ ( $r=0.9998$ ) 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 2.1.2.2.2.1 Independent laboratory validation

Comments of zRMS:	The method was evaluated by zRMS-Greece in RR of SAP50SCF (December 2023). <u>zRMS-Greece comments:</u> <i>The study is considered acceptable.</i>
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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 15:** 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 16:** Characteristics for the analytical method used for independent laboratory validation of folpet residues in animal matrices

	Phthalimide	
Specificity	Primary method	Confirmatory method
	LC – MS/MS m/z 148 → 130 blank value < 30 % LOQ	LC – MS/MS m/z 148 → 102 blank value < 30 % LOQ
Calibration (type, number of data points)	<u>Fat:</u> y=9.6e+004x+4.44e+003 (r=0.9997)  <u>Eggs:</u> y=9.28e+004x+3.18e+004 (r=0.9990)  <u>Milk:</u> y=9.77e+004x+3.93e+004 (r=0.9998)  6 data points	<u>Fat:</u> y=6.26e+004x+1.06e+004 (r=0.9998)  <u>Eggs:</u> y=6.07e+004x+5.69e+004 (r=0.9996)  <u>Milk:</u> 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/quantificati	LOQ = 0.01 mg/kg LOD = 0.003 mg/kg	

	<b>Phthalimide</b>
on	

## 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 2.1.2.2.2 Extraction efficiency

Not required. No further data has been provided.

### A 2.1.2.3 Description of analytical methods for the analysis of soil (KCP 5.2)

#### A 2.1.2.3.1 Analytical method

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

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 <sub>2</sub> O)	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  $\rightarrow$  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 (>  $\pm$  20%). Matrix matched standards were used for quantification for all matrices, by default.

LOQ: 0.01 mg/kg in all matrices.

**Table A 17: 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	<i>Quantification</i> Column: BEH C18 $m/z$ 146.0 $\rightarrow$ 41.9
		0.10	87.2	12.0	
		0.01	85.2	10.9	<i>Confirmation</i> Column: ZORBAX SB-C3 $m/z$ 146.0 $\rightarrow$ 41.9

**Table A 18: Characteristics for the analytical method used for validation of folpet residues in soil**

	Phthalimide Folpet	
Specificity	Primary method	Confirmatory method
	LC – MS/MS Column: BEH C18 $m/z$ 146.0 $\rightarrow$ 41.9	LC – MS/MS Column: ZORBAX SB-C3 $m/z$ 146.0 $\rightarrow$ 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

	Phthalimide <b>Folpet</b>
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 2.1.2.4 Description of Methods for the Analysis of Water (KCP 5.2)

### A 2.1.2.4.1 Analytical method 1

Comments of zRMS:	The method was evaluated by zRMS-Greece in RR of SAP50SCF (December 2023).  <u>zRMS-Greece comments:</u> <i>The GC-MS analytical method is acceptable and validated for the determination of folpet and phthalimide in drinking water.</i>
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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 19: 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	<i>Quantification</i> GC-MS, column: Optima-17 $m/z$ 146
		1	99.0	8.3	
		0.1	104.2	5.4	<i>Confirmation</i> GC-MS, column: DB-5 $m/z$ 146
		1	97.0	3.9	
	Phthalimide	0.1	74.2	2.2	<i>Quantification</i> GC-MS, column: Optima-17 $m/z$ 147
		1	74.6	4.1	
		0.1	82.6	7.7	<i>Confirmation</i> GC-MS, column: DB-5 $m/z$ 147
		1	76.4	7.1	

**Table A 20: Characteristics for the analytical method used for validation of folpet residues in water**

	Folpet	Phthalimide
Specificity	Primary method:	Primary method:



	GC-MS, column: Optima-17 $m/z$ 146 Confirmatory method: GC-MS, column: DB-5 $m/z$ 146	GC-MS, column: Optima-17 $m/z$ 147 Confirmatory method: GC-MS, column: DB-5 $m/z$ 147
Calibration (type, number of data points)	<u>Primary method:</u> $y=639.079x - 59.3437$ ( $r^2=0.997297$ )  <u>Confirmatory method:</u> $y=1091.88x - 67.0727$ ( $r^2=0.999768$ )  8 data points	<u>Primary method:</u> $y=15522.1x + 2825.76$ ( $r^2=0.998138$ )  <u>Confirmatory method:</u> $y=242.743x^2 + 2844.90x + 378.864$ ( $r^2=0.999583$ )  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 2.1.2.4.1.1 Independent laboratory validation

Comments of zRMS:	The method was evaluated by zRMS-Greece in RR of SAP50SCF (December 2023).
	<u>zRMS-Greece comments:</u> <i>The method is considered acceptable.</i>

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 21: 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	<i>Quantification</i> GC-MS, column: DB-17MS
		1.0	84	11	<i>m/z</i> 146
		0.10	78	5	<i>Confirmation</i> GC-MS, column: Optima 5 HT
		1.0	86	13	<i>m/z</i> 146
	Phthalimide	0.10	90	8	<i>Quantification</i> GC-MS, column: DB-17MS
		1.0	79	9	<i>m/z</i> 147
		0.10	82	7	<i>Confirmation</i> GC-MS, column: Optima 5 HT
		1.0	72	7	<i>m/z</i> 147

**Table A 22: 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 <i>m/z</i> 146 Confirmatory method: GC-MS, column: Optima 5 HT <i>m/z</i> 146	Primary method: GC-MS, column: DB-17MS <i>m/z</i> 147 Confirmatory method: GC-MS, column: Optima 5 HT <i>m/z</i> 147
Calibration (type, number of	<u>Primary method:</u>	<u>Primary method:</u>

data points)	$y=72909.6x - 1456.03$ ( $r^2=0.9918$ ) 8 data points  <u>Confirmatory method:</u> $y=4174.23x^2 + 16306.8x - 538.755$ $(r^2=0.9931)$ 7 data points	$y=2.11101e+006x + 574314$ ( $r^2=0.9977$ ) 9 data points  <u>Confirmatory method:</u> $y= - 28223.3x^2 + 1.4949e+006x + 237743$ $(r^2=0.9915)$ 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 2.1.2.5 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).  <u>zRMS-Greece comments:</u> <i>The GC-MS analytical method is acceptable and validated for the determination of folpet in air.</i>
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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 °C, 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/m<sup>3</sup>). 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/m<sup>3</sup> (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/m<sup>3</sup> (equivalent to 10.8 µg on sorbent material)

**Table A 23: 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 24: Characteristics for the analytical method used for validation of folpet and phthalimide residues in air**

	<b>Folpet</b>	<b>Phthalimide</b>
Specificity	GC-MS, column: Optima-17 <i>m/z</i> 146	GC-MS, column: Optima-17 <i>m/z</i> 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 found valid for the determination of folpet and phthalimide. All parameters are also according to the new guidance SANTE/2020/12830 rev. 2.

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

### A 2.1.2.6.1 Analytical method 1

Comments of zRMS:	<p>The method was evaluated by zRMS-Greece in RR of SAP50SCF (December 2023).</p> <p><u>zRMS-Greece comments:</u></p> <p><i>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.</i></p> <p><i>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.</i></p> <p><i>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.</i></p>
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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 25: 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	<i>Quantification</i> <i>m/z 148 → 130</i>
		0.05	83	6.2	<i>Confirmation</i> <i>m/z 148 → 102</i>

**Table A 26: Characteristics for the analytical method used for validation of folpet residues in urine**

	Phthalimide
Specificity	LC-MS/MS Primary method: <i>m/z</i> 148 → 130 Confirmatory method: <i>m/z</i> 148 → 102
Calibration (type, number of data points)	<u>Primary method:</u> $y=41719.3082x - 18584.5107$ ( $r=1.0000$ )  <u>Confirmatory method:</u> $y=25728.1308x - 14259.2504$ ( $r=1.0000$ )  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 2.1.2.7 Other Studies/ Information

No new or additional studies have been submitted.