

REGISTRATION REPORT

Part B

Section 5

Analytical Methods

Detailed summary of the risk assessment

Product code: GLOB2011I

Product name(s): SANKARI

Chemical active substance:

Pelargonic acid, 650 g/L

Central Zone

Zonal Rapporteur Member State: Poland

CORE ASSESSMENT/

(authorization)

Applicant: Globachem NV

Submission date: 31/07/2022

RMS Assessment: 15/01/2024

After commenting period: 05/05/2024

Update list studies: 28/05/2024

Version history

When	What
January 2024	RMS Assessment
May 2024	After commenting period
May 2024	Update list studies

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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 in the plant protection product.

Noticed data gaps are: none

RMS comments: The methods for the determination of residues in body fluids and tissues and ILV method for the determination of residues in drinking water are evaluated during the renewal process (RAR).

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)

The methods provided hereafter were not evaluated during the EU approval process of the active substance.

An overview on the acceptable methods and possible data gaps for analysis of pelargonic acid in plant protection product GLOB2011I is provided as follows:

Comments of zRMS:	Described method validation DNA7168 for the determination of concentrations of Active Substance pelargonic acid contained in GLOB2011I was validated in accordance with SANCO/3030/99 rev.5 and GLP. The method is acceptable and suitable for the determination of the active substance.
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Reference:	KCP 5.1.1
Report	Validation of the Methods of Determination of the Active Substance contained in GLOB2011I, in Compliance with Good Laboratory Practice, Pomeroy D., 2023c, Study # DNA7168
Guideline(s):	Yes, SANCO/3030/99 rev.5
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

The Sample Precision of Pelargonic Acid (C9) was performed using approximately 0.5g of the Formulation. The mass of the sample was accurately weighed into a 50mL volumetric flask, made to partial volume with Methanol and sonicated for 5 minutes. Once cooled to ambient temperature, the solution was made to final volume with Methanol.

500µL of this solution was transferred to a 25mL volumetric flask with 10µL of Boron trifluoride (13-15% Methanol complex) solution and 1mL of Tridecanoic acid Internal Standard (section 5.5). The volumetric flask was sealed with Parafilm tape and placed into an incubator set at 60°C for 2 hours to fully derivatize, then removed from the incubator and allowed to cool to room temperature (ambient) before being partially made to volume with Methanol and sonicated for 5 minutes. After cooling once more to room temperature (ambient), the volumetric flask was made to final volume with Methanol.

Six separate solutions were prepared following this methodology, then analysed using GC-FID under the following conditions:

GC-FID conditions:

Instrument: 6890 GC-FID
Column: ZB-Wax (30m x 0.25mm x 0.25µm)
Temperatures:
Column: 60°C held for 3 minutes, then 70°C/minute to 240°C, held for 5 minutes
Injector: 250°C
Carrier gas: Helium

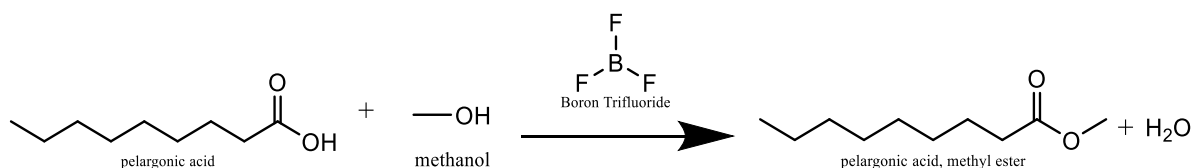
FID:
Heater Temperature: 250°C
Hydrogen Flow: 35mL/minute
Air Flow: 350mL/minute
Helium Flow: 20mL/minute

Data Collection: Chemstation
Retention Times: Pelargonic Acid (C9) approximately 10.7 to 10.8 minutes
Tridecanoic Acid Internal Standard approximately 18.0 minutes

GC-MSD conditions - Pelargonic Acid (C9) Derivatized MS Spectral Analysis:

Instrument: Shimadzu GC-MSD
Column: DB-XLB, (30m x 0.25mm x 0.25µm)
Temperatures:
Column: 40°C held for 2 minutes, then 20°C/minute to 300°C held for 5 minutes
Injector: 200°C
Split Ratio: Splitless
SCAN: 25-250m/z
Carrier Gas: Helium
Data Collection: GCMS Solutions
Retention Time: Pelargonic Acid (C9) approximately 9.2 minutes

It is known that the Derivatization of Pelargonic Acid with Boron Trifluoride in the presence of Methanol forms Pelargonic Acid, methyl ester.



LC-QTOF Conditions – Pelargonic Acid (C9) Non Derivatized MS Spectral Analysis:

LC Conditions:

Instrument: Agilent 1200 Series HPLC-DAD
Mode: Gradient Reverse Phase
Column: Agilent Poroshell 120 EC-C18 (150mm x 4.6mm)
Packing: EC-C18, 2.7µm
Eluent: A: Methanol
B: Deionised Water containing 0.05% (w/v) Ammonium Acetate
Flow Rate: 0.5mL/minute
Injection Volume: 5µL
Column Temperature: 30°C

Gradient Conditions:

Time (minutes)	Eluent A Percentage	Eluent B Percentage
0.00	53	47
18.0	90	10
19-30	53	47

Between 0.0-18.0 minutes, the ratio of the eluent is changing from 53% to 90% for eluent A and from 47% to 10% for eluent B. Between 18.0-19.0 minutes, the ratio of the eluent is changing from 90% to 53% for eluent A and from 10% to 47% for eluent B.

MS Conditions:

Instrument: Agilent 6500 Series QTOF Mass Spectrometer
Mode: Agilent Jetstream ESI
Ionisation: Negative
MS Scan Range: 50-1000m/z
MS/MS Scan Range: 20-500m/z
Extracted Ions: N/A Full Scan
Acquisition Rate: 1 Spectra/Second
Acquisition Time: 1000ms/Spectrum
Retention Time: Pelargonic Acid approximately 12.0 minutes

Gas Temperature: 300°C VCap: 3000V
Drying Gas Flow: 8L/minute Nozzle Voltage: 2000V
Nebulizer: 60psig Fragmentor: 100V
Sheath Gas: 300°C Skimmer: 65V
Sheath Gas Flow: 7L/minute OCT 1 RF Vpp: 750V
Collision Energy: 0, 5 10, 15, 20V

Data Acquisition: MassHunter

Validation - Results and discussions

Table 5.21.1-1: Methods suitable for the determination of active substance pelargonic acid in plant protection product GLOB2011I

	Pelargonic acid
Author(s), year	Pomeroy D., 2023

	Pelargonic acid
Principle of method	GC-FID
Linearity (linear between mg/L / % range of the declared content) (correlation coefficient, expressed as r)	Linearity of the analytical method was evaluated in a 0.01 to 0.4 mg/mL concentration range (corresponding to 50 to 2000 g a.s./L PPP concentration range). Seven concentration levels were used: 0, 0.01, 0.025, 0.1, 0.2, and 0.4 mg a.s./mL. Two replicate injections were done from each solution. The coefficient of determination (r^2) was calculated as 0.99415 (which is acceptable according to the acceptance criteria of SANCO/3030/99 rev.5 of > 0.99).
Precision – Repeatability Mean n = 6 (%RSD)	%RSD = 0.447 Hr = 0.312 SANCO/3030/99 rev.5 criteria are met (i.e. Horwitz %RSD less than 1.434 and Horrat (Hr) ≤ 1 at 637 g/L.
Accuracy - Recovery n = 6 (% Recovery)	<u>At 650 g/L:</u> Mean Recovery = 98.75% %RSD = 1.241 Hr = 0.866 SANCO/3030/99 rev.5 criteria are met (i.e. mean recovery between 97%-103%, Horwitz %RSD less than 1.432 and Horrat (Hr) ≤ 1 at 641.9 g/L. <u>At LOQ (125 g/L):</u> Mean Recovery = 101.4% %RSD = 1.498 Hr = 0.819 SANCO/3030/99 rev.5 criteria are met (i.e. mean recovery between 97%-103%, Horwitz %RSD less than 1.829 and Horrat (Hr) ≤ 1 at 126.7 g/L.
Interference/ Specificity	<u>Interference:</u> No interference ($>3\%$) and no significant peak present at the elution time of pelargonic acid. <u>Specificity:</u> the Spectra obtained for pelargonic acid confirm the species identification.
LOQ	LOQ = 125 g/L
Comment	-

Conclusion

Specificity, linearity, precision/repeatability, accuracy have been demonstrated as all acceptance criteria of SANCO/3030/99 rev.5 are all met. No interference was observed at the retention time of the active substance. The proposed method is thus suitable for the determination of pelargonic acid in GLOB2011I.

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

Not applicable. No relevant impurities listed in EFSA Journal 2013;11(1):3023.

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

Under current EU legislation methods on formulants are not required however if a formulant is defined as relevant for toxicity (environment, health) then a method needs to be provided. There are however no formulants in GLOB2011I that are defined as relevant for toxicity.

5.2.1.4 Applicability of existing CIPAC methods (KCP 5.1.1)

There are no CIPAC methods available for the determination of pelargonic acid in GLOB2011I.

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 pelargonic acid for the generation of pre-authorization data is given in the following table. For the detailed evaluation of new studies it is referred to Appendix 2.

Table 5.2-2: Validated methods for the generation of pre-authorization data

Component of residue definition: pelargonic acid				
Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing / EU agreed
Plants, plant products,... (Residues)	Primary	-	Not required	EFSA 2013
	Confirmatory (if required)	-	Not required	EFSA 2013
Animal products, food of animal origin,... (Residues)	Primary	-	Not required	EFSA 2013
	Confirmatory (if required)	-	Not required	EFSA 2013
Soil, water, sediment,... (Environmental fate)	Primary	-	Not required	EFSA 2013
	Confirmatory (if required)	-	Not required	EFSA 2013
Soil, water,... (Efficacy)	Primary	-	Not required	EFSA 2013
	Confirmatory (if required)	-	Not required	EFSA 2013
Feed, body fluids,... (Toxicology)	Primary	-	Not required	EFSA 2013
	Confirmatory (if required)	-	Not required	EFSA 2013
Body fluids, air,... (Exposure)	Primary	-	Not required	EFSA 2013
	Confirmatory (if required)	-	Not required	EFSA 2013
Sugar solution (50 % w/v su-crose solution + 0.1 % Xan-than) (Ecotoxicology)	Primary	8.1 mg a.s./L	HPLC-UV	Schabio S., 2022, Study No. 163761136
	Confirmatory (if required)	-	Not required	-

Component of residue definition: pelargonic acid				
Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing / EU agreed
Water stock solutions coming from bee larvae study (Ecotoxicology)	Primary	0.50 g a.s./L	HPLC-MS/MS	Coli M., 2022, Study No BT256/21.
	Confirmatory (if required)	-	Not required	-
Fortified test water coming from algae study (Ecotoxicology)	Primary	0.07 mg a.s./L	LC-MS	Börschig C., 2023a, Study No 167841210; Börschig C., 2023b, Study No 167841220; Börschig C., 2023c, Study 167841215
	Confirmatory (if required)	-	Not required	-
Fortified blank GLOB2011I diluted samples used in NTP studies. (Ecotoxicology)	Primary	22.5 g a.s./L	HPLC-UV	Bützler R., 2022a, Study No 167841086; Bützler R., 2022b, Study No 167841087
	Confirmatory (if required)	-	Not required	-

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.

Methods provided in point 5.2.1 can be used for post-authorization and monitoring purpose.

5.3.2 Description of analytical methods for the determination of residues of pelargonic acid (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 Renewal Assessment Report (incl. its addenda) the current legal residue definition is 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	No residue definition set	Not required	EFSA 2013
Plant, high acid content		Not required	EFSA 2013

Matrix	Residue definition	MRL / limit	Reference for MRL/level Remarks
Plant, high protein/high starch content (dry commodities)		Not required	EFSA 2013
Plant, high oil content		Not required	EFSA 2013
Plant, difficult matrices (hops, spices, tea)		Not required	EFSA 2013
Muscle	No residue definition set	Not required	EFSA 2013
Milk		Not required	EFSA 2013
Eggs		Not required	EFSA 2013
Fat		Not required	EFSA 2013
Liver, kidney		Not required	EFSA 2013
Soil (Ecotoxicology)	No residue definition set	Not required	EFSA 2013
Drinking water (Human toxicology)	No residue definition set	Not required	EFSA 2013
Surface water (Ecotoxicology)	No residue definition set	Not required	EFSA 2013
Air	No residue definition set	Not required	EFSA 2013
Tissue (meat or liver)	No residue definition set	Not required	EFSA 2013
Body fluids		Not required	EFSA 2013

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 pelargonic acid 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: no residue definition set (not required)				
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	-	Not required	EFSA 2013
	ILV	-	Not required	EFSA 2013
	Confirmatory (if required)	-	Not required	EFSA 2013
High acid content	Primary	-	Not required	EFSA 2013
	ILV	-	Not required	EFSA 2013
	Confirmatory	-	Not required	EFSA 2013

Component of residue definition: no residue definition set (not required)				
Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing / EU agreed
	(if required)			
High oil content	Primary	-	Not required	EFSA 2013
	ILV	-	Not required	EFSA 2013
	Confirmatory (if required)	-	Not required	EFSA 2013
High protein/high starch content (dry)	Primary	-	Not required	EFSA 2013
	ILV	-	Not required	EFSA 2013
	Confirmatory (if required)	-	Not required	EFSA 2013
Difficult (if required, depends on intended use)	Primary	-	Not required	EFSA 2013
	ILV	-	Not required	EFSA 2013
	Confirmatory (if required)	-	Not required	EFSA 2013

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:	-
Not required, because:	Not required because no residue definition is set and not analytical method is needed.

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 pelargonic acid 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: no residue definition set (not required)				
Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing
Milk	Primary	-	Not required	EFSA 2013
	ILV	-	Not required	EFSA 2013
	Confirmatory (if required)	-	Not required	EFSA 2013

Component of residue definition: no residue definition set (not required)				
Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing
Eggs	Primary	-	Not required	EFSA 2013
	ILV	-	Not required	EFSA 2013
	Confirmatory (if required)	-	Not required	EFSA 2013
Muscle	Primary	-	Not required	EFSA 2013
	ILV	-	Not required	EFSA 2013
	Confirmatory (if required)	-	Not required	EFSA 2013
Fat	Primary	-	Not required	EFSA 2013
	ILV	-	Not required	EFSA 2013
	Confirmatory (if required)	-	Not required	EFSA 2013
Kidney, liver	Primary	-	Not required	EFSA 2013
	ILV	-	Not required	EFSA 2013
	Confirmatory (if required)	-	Not required	EFSA 2013

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, because:	Not required because no residue definition is set and not analytical method is needed.

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 pelargonic acid 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: no residue definition set (not required)			
Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing
Primary	-	Not required	EFSA 2013
Confirmatory	-	Not required	EFSA 2013

For any special comments or remarkable points concerning the analytical methods for soil please refer to 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 pelargonic acid in surface and drinking water is given in the following tables. For the detailed evaluation of new/ additional studies it is referred to Appendix 2.

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

Component of residue definition: no residue definition set (not required)				
Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing
Drinking water	Primary	-	Not required	EFSA 2013
	ILV	-	Not required	EFSA 2013
	Confirmatory	-	Not required	EFSA 2013
Surface water	Primary	-	Not required	EFSA 2013
	Confirmatory	-	Not required	EFSA 2013

For any special comments or remarkable points concerning the analytical methods for water please refer to 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 pelargonic acid 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: no residue definition set (not required)			
Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing
Primary	-	Not required	EFSA 2013
Confirmatory	-	Not required	EFSA 2013

For any special comments or remarkable points concerning the analytical methods for air it is referred to 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 pelargonic acid 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: no residue definition set (not required)			
Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing
Primary	-	Not required	EFSA 2013
Confirmatory	-	Not required	EFSA 2013

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

5.3.2.8 Other studies/ information

In several ecotoxicological studies summarized in section B9 of the dRR, analytical methods were used for the detection of pelargonic acid applied as GLOB2011I in the different test mediums. The analytical part of these studies is summarized in Appendix 2.

Appendix 1 Lists of data considered in support of the evaluation

Tables considered not relevant can be deleted as appropriate.

MS to blacken authors of vertebrate studies in the version made available to third parties/public.

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	Pomeroy D.	2023c	Validation of the Methods of Determination of the Active Substance contained in GLOB2011I, in Compliance with Good Laboratory Practice Study number: DNA7168 David Norris Analytical Laboratories Ltd. GLP Unpublished	N	Globachem nv
KCP 5.2.1 Submitted as KCP 10.2.1	Börschig C.	2023a	GLOB2011I: Toxicity to Pseudokirchneriella subcapitata in an Algal Growth Inhibition Test Study number: 167841210 ibacon GmbH GLP Unpublished	N	Globachem nv
KCP 5.2.1 Submitted as KCP 10.2.1	Börschig C.	2023b	GLOB2011I: GLOB2011I: Acute Toxicity to Daphnia magna in a Semi-Static 48-hour Immobilisation Test Study number: 167841220 ibacon GmbH GLP Unpublished	N	Globachem nv
KCP 5.2.1 Submitted as KCP 10.2.1	Börschig C.	2023c	GLOB2011I: Toxicity to the Aquatic Plant Myriophyllum spicatum in a Semi-Static Growth Inhibition Test with a Prior Rooting Phase Study number: 167841215	N	Globachem nv

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
			ibacon GmbH GLP Unpublished		
KCP 5.2.1 Submitted as KCP 10.3.1.2	Schabio S.	2022	GLOB2011I: Chronic Oral Toxicity Test on the Honey Bee (<i>Apis mel-lifera</i> L.) in the Laboratory Study number: 163761136 ibacon GmbH GLP Unpublished	N	Globachem nv
KCP 5.2.1 Submitted as KCP 10.3.1.3	Colli M.	2022	Effects of GLOB2011I on honeybees (<i>Apis mellifera</i> L.) 22-day larval toxicity test with repeated exposure Study number: BT256/21 BioTecnologie BT S.r.l. GLP Unpublished	N	Globachem nv
KCP 5.2.1 Submitted as KCP 10.6	Bützler R.	2022a	GLOB2011I: Effects on Terrestrial (Non-Target) Plants: Seedling Emergence and Seedling Growth Test Study number: 167841086 ibacon GmbH GLP Unpublished	N	Globachem nv
KCP 5.2.1 Submitted as KCP 10.6	Bützler R.	2022b	GLOB2011I: Effects on Terrestrial (Non-Target) Plants: Vegetative Vigour Test Study number: 167841087 ibacon GmbH GLP Unpublished	N	Globachem nv

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
None					

The following tables are to be completed by MS

List of data submitted by the applicant and not relied on

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

Appendix 2 Detailed evaluation of submitted analytical methods

A 2.1 Analytical methods for pelargonic acid

A 2.2 Methods used for the generation of pre-authorization data (KCP 5.1)

Reference is made to 5.2.1 for a summary of the methods used to determine the active substance in the formulated product.

A 2.2.1.1.1 Method validation

Comments of zRMS:	Described method validation 167841220 for the determination of concentrations of pelargonic acid of the test item GLOB2011I in the test samples was validated in accordance with SANTE/2020/12830, Rev.1 and GLP. The method is acceptable and suitable for the determination of the active substance.
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Reference:	KCP 5.2.1
Report	GLOB2011I: GLOB2011I: Acute Toxicity to Daphnia magna in a Semi-Static 48-hour Immobilisation Test, Börschig C., 2023a, Study No 167841220
Guideline(s):	SANTE/2020/12830, Rev.1 (24/02/2021)
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

In the course of this study an LC-MS analytical method has been validated for the analysis of the test item in fortified test water.

Apparatus

Analytical balance:	Mettler Toledo and/or Sartorius
Pipettes:	Eppendorf, various models
Dispensers:	Brand, various models
Ultrasonic bath:	Sonorex Digitec DT, Bandelin
Standard laboratory equipment:	Magnetic stirrers (e.g. MIX eco 15, 2magnetic motion), Vortex apparatus (e.g. Phoenix instruments) etc.
Laboratory glass ware:	Volumetric flasks, beakers, Pasteur pipettes, graduate cylinders, glass bottles etc.; different suppliers
Autosampler vials:	1.5 mL volume with PTFE sealed screw caps and septum, VWR
Centrifuge:	Thermo Fisher Scientific and/or Heraeus

Chromatographic conditions

LC:	Agilent Series 1290 pump and autosampler
Column:	PerfectSil 120 ODS-2, 125 * 3 mm, 5 µm
Temperature:	37 °C
Mobile Phase:	90% A: Methanol containing 0.05 % acetic acid

Run Time: 10% B: HPLC water containing 0.05 % acetic acid
5.5 min
Flow Rate: 0.5 mL/min
Injection Volume: 20 ~~μL~~ 10 μL
Detector: Mass spectrometer API 4000

Parameters:

Ion Source: Electrospray ionization (ESI) negative
Curtain gas (CUR): 25 psi
Nebulizer gas (GS1): 60 psi
Voltage (IS): -4500 V
Heater Temperature (TEM) : 600 °C
Turbo gas (GS2): 70 psi
Dwell time: 300 ms

Mass:

Item	Ion	Declustering Potential (DP)	Entrance Potential (EP)
	[m/z]	[V]	[V]
quantifier	156.965	-30	-9

Integration Software: Analyst Version 1.6.2

Standard Solutions used for Quantification and Matrix Effect Determination

Stock Solution: Approximately 10 mg of the analytical reference item were dissolved in 10 mL acetonitrile to obtain a stock solution of approximately 1 g reference item /L.

Standard Solutions for Quantification: Appropriate amounts of the stock solution were diluted with test water/(ACN + 0.1% HAc) 1/1 (v/v) to obtain standard solutions in the range from 0.01 – 0.35 mg reference item/L. The final solvent composition of the standard solutions is matrix-matched, i.e. comparable to the final solvent composition of the fortified and biological samples. Exact data were documented in the raw data.

Non-Matrix Matched Standard Solutions for Matrix Effect Determination: Appropriate amounts of the stock solution were diluted with HPLC water/(ACN + 0.1% HAc) 1/1 (v/v) to obtain standard solutions at a concentration of 0.02 and 0.3 mg reference item/L (non-matrix matched). Exact data were documented in the raw data.

Analytical sample

Fortified Samples: Approximately 50 mg of the test item were homogenously dispersed (5 minutes ultrasonication, 60 minutes stirring) in 50 mL test water/(ACN + 0.1% HAc) 1/1 (v/v) to obtain a stock solution of approximately 1 g test item/L. Exact weighing and purity of the test item was considered for results evaluation. Two independent stock solutions were prepared. Appropriate amounts of these stock solutions were diluted with test water/(ACN + 0.1% HAc) 1/1 (v/v) to obtain intermediate dilutions of 20 mg test item/L. The necessary solutions were then diluted further with test water to obtain fortified samples at a level of 0.2 and 60 mg test item/L. Exact values were documented in the raw data.

Blank Control Samples: Two replicates were prepared by using untreated test water.

Sample Preparation

Fortified samples and analytical blank control samples:

The samples were shaken well and were diluted with acetonitrile containing 0.1% acetic acid by factor 2. They were diluted further with test water/(ACN + 0.1% HAc) 1/1 (v/v) to match the calibration range, if necessary.

Results and discussions

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

Matrix	Analyte	Fortification level (g test item/L) (n = 5-8)	Mean recovery (%)	RSD (%)
Fortified test water coming from daphnia magna study performed with GLOB2011I	Pelargonic acid	0.2 (n=5)	98	3
		60 (n = 5)	88	2

Table A 2: Characteristics for the analytical method used for validation of pelargonic acid residues in fortified test water and test media coming from algae study performed with GLOB2011I

	Pelargonic acid
Specificity	No significant (< 30%) interference of total peak area for the target analyte was found with respect to the LOQ level. The representative standard, blank control and fortification chromatograms show no significant interfering signals at the retention time of the analyte (Figures are provided). The mass spectrum demonstrates the assigning of the chosen mass of m/z 156.965 (quantifier) to the target analyte. In summary, selectivity and specificity criteria set forth by SANTE/2020/12830 rev. 1 were fulfilled.
Calibration (type, number of data points)	Linear r ² ≥ 0.999 number of data points: 10
Calibration range	0.01 to 0.35 mg a.s./L
Assessment of matrix effects is presented	Yes. A significant matrix effect of overall -75 % was determined for pelargonic acid in matrix compared to pure solvent. Independently of the determined matrix effect, matrix-matched calibration standards were used for quantification
Limit of quantification	0.2 mg itest item/L corresponding to 0.07 mg a.s./L after dilution by factor 2
Limit of Detection	0.01 mg a.s./L
Standard and Extract Stability:	Storage stability of final extracts and standard solutions was not investigated since all prepared samples were not stored between end of sample preparation and beginning of analysis.

Conclusion

The method was sufficiently validated according to SANTE/2020/12830 Rev.1. and can be used to reliably and accurately determine pelargonic acid in fortified test media coming from algae study performed with GLOB2011I.

Comments of zRMS:	Described method 167841220 for the determination of concentrations of pelargonic acid of the test item GLOB2011I in the test samples was validated in accordance with SANTE/2020/12830, Rev.1 and GLP. The method is acceptable and suitable for the determination of the active substance.
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Reference:	KCP 5.2.1
Report	GLOB2011I: Toxicity to <i>Pseudokirchneriella subcapitata</i> in an Algal Growth Inhibition Test, Börschig C., 2023b, Study No 167841210
Guideline(s):	SANTE/2020/12830, Rev.1 (24/02/2021)
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

In the course of this study an LC-MS analytical method has been validated for the analysis of the test item in fortified test water.

Apparatus

Analytical balance:	Mettler Toledo and/or Sartorius
Pipettes:	Eppendorf, various models
Dispensers:	Brand, various models
Ultrasonic bath:	Sonorex Digitec DT, Bandelin
Standard laboratory equipment:	Magnetic stirrers (e.g. MIX eco 15, 2magnetic motion), Vortex apparatus (e.g. Phoenix instruments) etc.
Laboratory glass ware:	Volumetric flasks, beakers, Pasteur pipettes, graduate cylinders, glass bottles etc.; different suppliers
Autosampler vials:	1.5 mL volume with PTFE sealed screw caps and septum, VWR
Centrifuge:	Thermo Fisher Scientific and/or Heraeus

Chromatographic conditions

LC:	Agilent Series 1290 pump and autosampler
Column:	PerfectSil 120 ODS-2, 125 * 3 mm, 5 µm
Temperature:	37 °C
Mobile Phase:	90% A: Methanol containing 0.05 % acetic acid 10% B: HPLC water containing 0.05 % acetic acid
Run Time:	5.5 min
Flow Rate:	0.5 mL/min
Injection Volume:	20 µL
Detector:	Mass spectrometer API 4000
<u>Parameters:</u>	
Ion Source: Electrospray ionization (ESI) negative	
Curtain gas (CUR): 25 psi	

Nebulizer gas (GS1): 60 psi
Voltage (IS): -4500 V
Heater Temperature (TEM) : 600 °C
Turbo gas (GS2): 70 psi
Dwell time: 300 ms
Mass:

Item	Ion	Declustering Potential (DP)	Entrance Potential (EP)
	[m/z]	[V]	[V]
quantifier	156.965	-30	-9

Integration Software: Analyst Version 1.6.2

Standard Solutions used for Quantification and Matrix Effect Determination

Stock Solution: Approximately 10 mg of the analytical reference item were dissolved in 10 mL acetonitrile (1 minute ultrasonication) to obtain a stock solution of approximately 1 g reference item /L.

Standard Solutions for Quantification: Appropriate amounts of the stock solution were diluted with test water/(ACN + 0.1% HAc) 1/1 (v/v) to obtain standard solutions in the range from 0.01 – 0.35 mg reference item/L. The final solvent composition of the standard solutions is matrix-matched, i.e. comparable to the final solvent composition of the fortified samples. Exact data were documented in the raw data.

Non-Matrix Matched Standard Solutions for Matrix Effect Determination: Appropriate amounts of the stock solution were diluted with HPLC water/(ACN + 0.1% HAc) 1/1 (v/v) to obtain standard solutions at a concentration of 0.02 and 0.3 mg reference item/L (non-matrix matched). Exact data were documented in the raw data.

Analytical sample

Fortified Samples: Approximately 50 mg of the test item were homogenously dispersed (5 minutes ultrasonication, 50 minutes stirring) in 50 mL test water/(ACN + 0.1% HAc) 1/1 (v/v) to obtain a stock solution of approximately 1 g test item/L. Exact weighing and purity of the test item was considered for results evaluation. Two independent stock solutions were prepared. Appropriate amounts of these stock solutions were diluted with test water/(ACN + 0.1% HAc) 1/1 (v/v) to obtain intermediate dilutions of 50 and 1 mg test item/L. The necessary solutions were then diluted further with test water to obtain fortified samples at a level of 0.2 and 300 mg test item/L. Exact values were documented in the raw data.

Blank Control Samples: Two replicates were prepared by using untreated test water.

Sample Preparation

Fortified samples and analytical blank control samples:

The samples were shaken well and were diluted with acetonitrile containing 0.1% acetic acid by factor 2. They were diluted further with test water/(ACN + 0.1% HAc) 1/1 (v/v) to match the calibration range, if necessary. The samples were centrifuged (13000 rpm, 3 minutes) before analysis.

Results and discussions

Table A 3: Recovery results from method validation of pelargonic acid using the analytical method

Matrix	Analyte	Fortification level (g test item/L) (n = 5-8)	Mean recovery (%)	RSD (%)
Fortified test water coming from algae study performed with GLOB2011I	Pelargonic acid	0.2 (n = 5)	119	2
		300 (n = 5)	103	2
Overall mean value (n = 10)			111	8

Table A 4: Characteristics for the analytical method used for validation of pelargonic acid residues in test media coming from algae study performed with GLOB2011I

	Pelargonic acid
Specificity	No significant (< 30%) interference of total peak area for the target analyte was found with respect to the LOQ level. The representative standard, blank control and fortification chromatograms show no significant interfering signals at the retention time of the analyte (Figures are provided). The mass spectrum demonstrates the assigning of the chosen mass of m/z 156.965 (quantifier) to the target analyte. In summary, selectivity and specificity criteria set forth by SANTE/2020/12830 rev. 1 were fulfilled.
Calibration (type, number of data points)	Linear $r^2 \geq 0.99$ number of data points: 9
Calibration range	0.02 to 0.35 mg a.s./L
Assessment of matrix effects is presented	Yes. A significant matrix effect of overall -45 % was determined for pelargonic acid in matrix compared to pure solvent. Independently of the determined matrix effect, matrix-matched calibration standards were used for quantification.
Limit of quantification	0.2 g itest item/L corresponding to 0.0716 mg a.s./L after dilution by factor 2
Limit of Detection	0.02 mg a.s./L
Standard and Extract Stability:	Storage stability of final extracts and standard solutions was not investigated since all prepared samples were not stored between end of sample preparation and beginning of analysis.

Conclusion

The method was sufficiently validated according to SANTE/2020/12830 Rev.1. and can be used to reliably and accurately determine pelargonic acid in test media coming from algae study performed with GLOB2011I.

Comments of zRMS: Described method 167841215 for the determination of concentrations of pelargon-

	ic acid of the test item GLOB2011I in the test samples was validated in accordance with SANTE/2020/12830, Rev.1 and GLP. The method is acceptable and suitable for the determination of the active substance.
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Reference:	KCP 5.2.1
Report	GLOB2011I: Toxicity to the Aquatic Plant <i>Myriophyllum spicatum</i> in a Semi-Static Growth Inhibition Test with a Prior Rooting Phase, Börschig C., 2023c, Study 167841215
Guideline(s):	SANTE/2020/12830, Rev.1 (24/02/2021)
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

In the course of this study an LC-MS analytical method has been validated for the analysis of the test item in fortified test water.

Apparatus

Analytical balance:	Mettler Toledo and/or Sartorius
Pipettes:	Eppendorf, various models
Dispensers:	Brand, various models
Ultrasonic bath:	Sonorex Digitec DT, Bandelin
Standard laboratory equipment:	Magnetic stirrers (e.g. MIX eco 15, 2magnetic motion), Vortex apparatus (e.g. Phoenix instruments) etc.
Laboratory glass ware:	Volumetric flasks, beakers, Pasteur pipettes, graduate cylinders, glass bottles etc.; different suppliers
Autosampler vials:	1.5 mL volume with PTFE sealed screw caps and septum, VWR
Centrifuge:	Thermo Fisher Scientific and/or Heraeus

Chromatographic conditions

LC:	Agilent Series 1200 or 1290 pump and autosampler
Column:	PerfectSil 120 ODS-2, 125 * 3 mm, 5 µm
Temperature:	37 °C
Mobile Phase:	On Agilent Series 1200 pump: 10% A: HPLC water containing 0.05 % acetic acid 90% B: Methanol containing 0.05 % acetic acid On Agilent Series 1290 pump: 90% A: Methanol containing 0.05 % acetic acid 10% B: HPLC water containing 0.05 % acetic acid
Run Time:	5.5 min
Flow Rate:	0.5 mL/min
Injection Volume:	20 µL
Detector:	Mass spectrometer API 4000
	<u>Parameters:</u>
	Ion Source: Electrospray ionization

(ESI) negative
 Curtain gas (CUR): 25 psi
 Nebulizer gas (GS1): 60 psi
 Voltage (IS): -4500 V
 Heater Temperature (TEM) : 600 °C
 Turbo gas (GS2): 70 psi
 Dwell time: 300 ms
Mass:

Item	Ion	Declustering Potential (DP)	Entrance Potential (EP)
	[m/z]	[V]	[V]
quantifier	156.965	-30	-9

Integration Software: Analyst Version 1.6.2

Standard Solutions used for Quantification and Matrix Effect Determination

Stock Solution: Approximately 10 mg of the analytical reference item were dissolved in 10 mL acetonitrile (up to 5 minutes ultrasonication) to obtain a stock solution of approximately 1 g reference item /L. Exact weighing and purity of the reference item was considered for results evaluation.

Standard Solutions for Quantification: Appropriate amounts of the stock solution were diluted with test water/(ACN + 0.1% HAc) 1/1 (v/v) to obtain standard solutions in the range from 0.01 – 0.35 mg reference item/L. The final solvent composition of the standard solutions is matrix-matched, i.e. comparable to the final solvent composition of the fortified and biological samples. Exact data were documented in the raw data.

Non-Matrix Matched Standard Solutions for Matrix Effect Determination: Appropriate amounts of the stock solution were diluted with HPLC water/(ACN + 0.1% HAc) 1/1 (v/v) to obtain standard solutions at a concentration of 0.02 and 0.3 mg reference item/L (non-matrix matched). Exact data were documented in the raw data.

Analytical sample

Fortified Samples: Approximately 50 mg of the test item were homogenously dispersed (5 minutes ultrasonication, 60 minutes stirring) in 50 mL test water/(ACN + 0.1% HAc) 1/1 (v/v) to obtain a stock solution of approximately 1 g test item/L. Exact weighing and purity of the test item was considered for results evaluation. Five independent stock solutions were prepared. Appropriate amounts of these stock solutions were diluted with test water/(ACN + 0.1% HAc) 1/1 (v/v) to obtain intermediate dilutions of 100, 50, 10 and/or 1 mg test item/L. The necessary solutions were then diluted further with test water to obtain fortified samples at a level of 0.2 and 300 mg test item/L. Exact values were documented in the raw data.

Blank Control Samples: Two replicates were prepared by using untreated test water.

Sample Preparation

Fortified samples and analytical blank control samples:

The samples were shaken well and were diluted with acetonitrile containing 0.1% acetic acid by factor 2. They were diluted further with test water/(ACN + 0.1% HAc) 1/1 (v/v) to match the calibration range, if necessary.

Results and discussions

Table A 5: Recovery results from method validation of pelargonic acid using the analytical method

Matrix	Analyte	Fortification level (g test item/L) (n = 5-8)	Mean recovery (%)	RSD (%)
Fortified test water coming from Myriophyllum study performed with GLOB2011I	Pelargonic acid	0.2 (n=9)	91	13
		300 (n = 15)	87	12
Overall mean value (n = 24)			89	12

Table A 6: Characteristics for the analytical method used for validation of pelargonic acid residues in fortified test water and test media coming from aquatic plant study performed with GLOB2011I

	Pelargonic acid
Specificity	No significant (< 30%) interference of total peak area for the target analyte was found with respect to the LOQ level. The representative standard, blank control and fortification chromatograms show no significant interfering signals at the retention time of the analyte (Figures are provided). The mass spectrum demonstrates the assigning of the chosen mass of m/z 156.965 (quantifier) to the target analyte. In summary, selectivity and specificity criteria set forth by SANTE/2020/12830 rev. 1 were fulfilled.
Calibration (type, number of data points)	Linear $r^2 \geq 0.99$ number of data points: 10
Calibration range	0.01 to 0.35 mg a.s./L
Assessment of matrix effects is presented	Yes. A significant matrix effect of overall -35 % was determined for pelargonic acid in matrix compared to pure solvent. Independently of the determined matrix effect, matrix-matched calibration standards were used for quantification
Limit of quantification	0.2 mg itest item/L corresponding to 0.07 mg a.s./L after dilution by factor 2
Limit of Detection	0.01 mg a.s./L
Standard and Extract Stability:	Storage stability of final extracts and standard solutions was not investigated since all prepared samples were not stored between end of sample preparation and beginning of analysis.

Conclusion

The method was sufficiently validated according to SANTE/2020/12830 Rev.1. and can be used to reliably and accurately determine pelargonic acid in test media coming from aquatic plant study performed with GLOB2011I.

Comments of zRMS: Described method 163761136 for the determination of concentrations of pelargon-

	ic acid of the test item GLOB2011I in the test samples was validated in accordance with SANTE/2020/12830, Rev.1 and GLP. The method is acceptable and suitable for the determination of the active substance.
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Reference:	KCP 5.2.1
Report	GLOB2011I: Chronic Oral Toxicity Test on the Honey Bee (<i>Apis mellifera</i> L.) in the Laboratory, Schabio S., 2022, Study No 163761136.
Guideline(s):	SANTE/2020/12830 Rev.1. (24/02/2021)
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

In the course of this study an HPLC-UV analytical method has been validated for the analysis of the test item in a sugar solution (50 % w/v sucrose sol. + 0.1 % xanthan).

HPLC-UV Conditions:

System:	Waters Acquity		
Software:	Empower 3		
Column:	Acquity UPLC BEH C18, 50 x 2.1 mm; 1.7 µm		
Temperature:	60 °C		
Mobile Phase:	A: HPLC-water + 0.2 % H ₃ PO ₄ B: acetonitrile		
Gradient Mode:	Time [min]	Solvent A [%]	Solvent B [%]
	initial	70	30
	0.33	70	30
	2.0	45	55
	2.1	5	95
	3.2	5	95
	3.3	70	30
	5.0	70	30
Total Run Time:	5.0 minutes		
Flow Rate:	0.5 mL/min		
Injection Volume:	10 µL		
Detector:	DA- Detector at 191 to 400 nm, monitoring wavelength at 209 nm		
Retention Time:	Pelargonic Acid	2.4 minutes	

Sample Preparation

Sample Preparation:	After thawing an aliquot of each sample was diluted with solvent mixture to match the calibration range. Samples were diluted while solutions were stirring, if necessary. The samples were analysed on the day of processing.
Fortification Procedure:	The test item was dissolved in 50 % w/v sucrose solution + 0.1 % Xanthan (stirred for 30 minutes) to get fortified samples of about 12 g test item/L. These solutions were diluted in 50 % w/v sucrose solution + 0.1 % Xanthan to obtain fortified samples at approximately 0.24 g test item/L.
Replicates:	Five independent replicates per fortification level Two independent replicates of matrix blanks

Results and discussions

Table A 7: Recovery results from method validation of pelargonic acid using the analytical method

Matrix	Analyte	Fortification level (mg test item/L) (n = 5)	Mean recovery (%)	RSD (%)
Sugar solution (50 % w/v sucrose solution + 0.1 % Xanthan)	Pelargonic acid	0.24	92	8
		120	82	6

Table A 8: Characteristics for the analytical method used for validation of pelargonic acid residues in sugar solution (50 % w/v sucrose solution + 0.1 % Xanthan)

	Pelargonic acid
Specificity	No interference above 30% of LOQ was observed in control samples at retention time of target analyte.
Calibration (type, number of data points)	Linear $r^2 \geq 0.999$ number of data points: 7
Calibration range	2.0 to 40 mg pelargonic acid/L corresponding to 25 % of LOQ to 123 % of higher fortification level
Assessment of matrix effects is presented	Yes. No significant matrix effect (≤ 20 %) was observed (matrix effect = 1%).
Limit of quantification	0.24 g test item/L corresponding to 8.1 mg Pelargonic Acid/L after dilution by factor 20
Limit of Detection	2.0 mg Pelargonic Acid/L
Standard and Extract Stability:	The stock solution of the reference item was prepared on the day of analysis. Samples were analysed on the day of sample processing. Therefore, stability of standards and extract were not assessed.

Conclusion

The method was sufficiently validated according to SANTE/2020/12830 Rev.1. and can be used to reliably and accurately determine pelargonic acid in sugar solution (50 % w/v sucrose solution + 0.1 % Xanthan).

Comments of zRMS:	Described method BT256/21 for the determination of concentrations of pelargonic acid of the test item GLOB2011I in the test samples was validated in accordance with SANTE/2020/12830, Rev.1 and GLP. The method is acceptable and suitable for the determination of the active substance.
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Reference: KCP 5.2.1

Report Effects of GLOB2011I on honeybees (*Apis mellifera* L.) 22-day larval toxicity test with repeated exposure, Coli M., 2022, Study No BT256/21.

Guideline(s): SANTE/2020/12830, Rev.1 (24/02/2021)

Deviations: No

GLP: Yes

Acceptability: Yes

Materials and methods

In the course of this study an HPLC-MS/MS analytical method has been validated for the analysis of the test item in an ultra-pure water solution.

Apparatus

Liquid Chromatograph: Agilent LC-MS/MS System: UHPLC 1290 series with 6495b Triple Quad. Spectrometer
Data analysis software: Mass Hunter Quantitative Analysis for QQQ, version B.08.00 - Agilent Technologies - 2016
Columns: Agilent Zorbax RRHD eclipse Plus C18 2.1 x 50 mm – 1.8 µm
Phenomenex Kinetex 1.7 µm Biphenyl 100A 100* 2.1 mm (for the confirmatory analysis)
Analytical balance: Mettler Toledo XP105DR
Pipettes: Gilson 10-5000 µL
Routine laboratory glassware

Chromatographic conditions

Eluent A: UP water acidified with 0.1% with acetic acid
Eluent B: methanol acidified with 0.1% with acetic acid

Eluent ratio A/B:

Time	% A	% B
5 min	5.0	95.0
6 min	5.0	95.0
6.1 min	60.0	40.0

Eluent ratio A/B in the confirmatory analysis:

Time	% A	% B
5 min	20.0	80.0
6 min	20.0	80.0
6.1 min	60.0	40.0

Flow rate: 0.4 mL/min
Injection volume: 1.0 µL
Column temperature: 40°C
Run time: 7.5 minutes
Retention time: 3.6 minutes
4.4 minutes in the confirmatory analysis

Detector parameters

Scan Type: MS2 SIM
Ion source: ESI (Electron Spray Ionization) + Agilent Jet Stream
Gas temperature: 200°C
Gas flow: 14 L/min.
Nebulizer: 20 psi
Capillary: 3000V
Polarity: Negative
Delta EMV: 0
Mass: 157.1
Dwell: 200
Frag (V): 380
Cell acc (V): 5

Sample Preparation

The stock solutions for limit of detection (LOD) and Calibration were prepared with the analytical standard and described as follows:

Solution code	Amount	Final volume [mL]	Analyte concentration
SS1	11.4 mg of Pelargonic acid analytical standard	10 of acetonitrile	1138.86 mg/L
SS2	0.9 mL of SS1	10 of UP water	102.4974 mg/L

The fortified sample solutions for the recovery and repeatability test were prepared as described below.

REC HIGH solutions:

Five solutions were prepared by weighing about 450 g of test item and made up to the volume of 10 mL with UP water, obtaining the REC HIGH solutions. The REC HIGH solutions were diluted with UP water as following described and analyzed by single injection: 0.15 mL to 10 mL and then 0.1 mL to 10 mL (dilution factor = 6666.67).

LOQ solutions:

0.165 mL of REC HIGH 1 were diluted in a total of 10 mL with UP water. Five solutions were prepared obtaining the LOQ solutions. The LOQ solutions were diluted with UP water as following described and analyzed by single injection: 0.35 mL to 10 mL and then 0.1 mL to 1.0 mL (dilution factor = 285.71).

Results and discussions

Table A 9: Recovery results from method validation of pelargonic acid using the analytical method

Matrix	Analyte	Fortification level (g/L) (n = 5)	Mean recovery (%)	RSD (%)
Water stock solutions coming from bee larvae study performed with GLOB2011I	Pelargonic acid	0.5	92.29 87,00	3.51
		30	98.36 92,72	7.26

Table A 10: Characteristics for the analytical method used for validation of pelargonic acid residues in water stock solutions coming from bee larvae study performed with GLOB2011I

	Pelargonic acid
Specificity	The method used is highly specific (HPLC-MS/MS, ion acquired with a mass of 157.1). No signal higher than 30% of the lowest fortified level (LOQ) was detected at the retention time of pelargonic acid in the untreated matrix.
Calibration (type, number of data points)	Linear $r^2 \geq 0.999$ number of data points: 5
Calibration range	512.487 to 5637.357 µg/L corresponding to undiluted concentrations in water of 0.1464 to 37.5824 g/L.
Assessment of matrix effects is presented	No. The matrix effect was not evaluated because all the analysed solutions were prepared in ultra pure water.
Limit of quantification	0.50 g/L (nominal), 0.4568 g/L (mean measured)
Limit of Detection	512.5 µg/L

	Pelargonic acid
Standard and Extract Stability:	The standard solutions were prepared fresh and used within 24 hours from preparation so no stability check was carried out.

Conclusion

The method was sufficiently validated according to SANTE/2020/12830 Rev.1. and can be used to reliably and accurately determine pelargonic acid in water sock solutions coming from bee larvae study performed with GLOB2011I.

Comments of zRMS:	Described method 167841086 for the determination of concentrations of pelargonic acid of the test item GLOB2011I in the test samples was validated in accordance with SANTE/2020/12830, Rev.1 and GLP. The method is acceptable and suitable for the determination of the active substance.
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Reference:	KCP 5.2.1
Report	GLOB2011I: Effects on Terrestrial (Non-Target) Plants: Seedling Emergence and Seedling Growth Test, Bützler R., 2022a, Study No 167841086
Guideline(s):	SANTE/2020/12830, Rev.1 (24/02/2021)
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

In the course of this study an HPLC-UV analytical method has been validated for the analysis of the test item in aqueous solutions.

Apparatus

Analytical balance:	Mettler Toledo and/or Sartorius
Pipettes:	Eppendorf, various models
Dispensers:	Brand, various models
Ultrasonic bath:	Sonorex Digitec DT, Bandelin
Standard laboratory equipment:	Magnetic stirrers (e.g. MIX eco 15, 2magnetic motion), Vortex apparatus (e.g. Phoenix instruments) etc.
Laboratory glass ware:	Volumetric flasks, beakers, Pasteur pipettes, graduate cylinders, glass bottles etc.; different suppliers
Autosampler vials:	1.5 mL volume with PTFE sealed screw caps and septum, VWR
Centrifuge:	Thermo Fisher Scientific and/or Heraeus

Chromatographic conditions

System:	Waters Acquity		
Software:	Empower 3		
Column:	Acquity UPLC BEH, C18 50 x 2.1 mm; 1.7 µm		
Temperature:	60°C		
Mobile Phase:	A: HPLC-water + 0.2% phosphoric acid B: acetonitrile		
Gradient Mode:	Time [min]	Solvent A [%]	Solvent B [%]
	initial	70	30

0.33	70	30
2.00	45	55
2.10	5	95
3.20	5	95
3.30	70	30
5.00	70	30

Total Run Time: 5 minutes
Flow Rate: 0.5 mL/min
Injection Volume: 10 µL
Detector: DA- Detector at 191 to 400 nm,
monitoring wavelength at 209 nm
Retention Time: Pelargonic acid: 2.45 minutes

Standard Solutions used for Quantification

Stock Solution: The reference item was dissolved with acetonitrile to obtain a stock solution of approximately 1 g reference item/L. The stock solution was prepared freshly on the day of analysis.

Solvent Mixture: Acetonitrile/pure water (50/50 v/v)

Standard Solutions: Aliquots of the stock solution was diluted with pure water (diluted by factor 300 in solvent mixture) to get matrix matched standard solutions in the range from 15 to 150 mg /L for each reference item.

For evaluation of possible matrix effects solvent standards of 15 mg reference item /L and 150 mg reference item /L were prepared in solvent mixture.

Sample

Duplicate samples of approximately 10 mL from the homogenous stock solution and two samples of the matrix (deionised water) as control solution were taken from the biological ibacon study 167841087. The concentration of the active ingredient pelargonic acid was determined in one sample of each duplicate.

Sample Preparation

Sample Preparation: After thawing an aliquot of each sample was diluted with solvent mixture to match the calibration range.

The samples were diluted, some solutions of them stirred in the process. The samples were analysed on the day of processing.

Fortification Procedure: The test item was dissolved in pure water (stirred for 30 minutes) to get fortified samples of about 45 g test item/L. These solutions were diluted with pure water to obtain fortified samples of about 22.5 g test item/L.

Afterwards fortified samples were diluted with solvent mixture to match calibration range.

Replicates: Five independent replicates per fortification level.

Two independent replicates of matrix blanks.

Results and discussions

Table A 11: Recovery results from method validation of pelargonic acid using the analytical method

Matrix	Analyte	Fortification level (g test item/L) (n = 5)	Mean recovery (%)	RSD (%)
Fortified blank GLOB2011I diluted samples.	Pelargonic acid	22.5 (n=5)	94	7
		45 (n = 5)	91	4
Overall mean value (n = 10)			93	6

Table A 12: Characteristics for the analytical method used for validation of pelargonic acid residues in fortified blank GLOB2011I diluted samples

	Pelargonic acid
Specificity	No interference above 30% of LOQ was observed in control samples at retention time of target analyte. The representative standard, blank control and fortification chromatograms show no significant interfering signals at the retention time of the analyte (Figures are provided).
Calibration (type, number of data points)	Linear $r^2 \geq 0.999$ number of data points: 8
Calibration range	15 to 150 mg pelargonic acid /L corresponding to 28% of LOQ to 140% of higher fortification level
Assessment of matrix effects is presented	Yes. No significant matrix effect ($\leq 20\%$) was observed. Matrix effect = 0% (15 mg a.s./L) and 1% (150 mg a.s./L) However, matrix-matched standards were used for determination of the active ingredients.
Limit of quantification	22.5 g a.s./L
Limit of Detection	15 mg a.s./L
Standard and Extract Stability:	The stock solution of the reference item was prepared on the day of analysis. Samples were analysed on the day of sample processing. Therefore, stability of standards and extract were not assessed.

Conclusion

The method was sufficiently validated according to SANTE/2020/12830 Rev.1. and can be used to reliably and accurately determine pelargonic acid in aqueous solutions as used in the seedling emergence test.

Comments of zRMS:	Described method 167841087 for the determination of concentrations of pelargonic acid of the test item GLOB2011I in the test samples was validated in accordance with SANTE/2020/12830, Rev.1 and GLP. The method is acceptable and suitable for the determination of the active substance.
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Reference: KCP 5.2.1

Report GLOB2011I: Effects on Terrestrial (Non-Target) Plants: Vegetative Vigour Test, Bützler R., 2022b, Study No 167841087

Guideline(s): SANTE/2020/12830, Rev.1 (24/02/2021)

Deviations: No

GLP: Yes

Acceptability: Yes

Materials and methods

In the course of this study an HPLC-UV analytical method has been validated for the analysis of the test item in aqueous solutions.

Apparatus

Analytical balance: Mettler Toledo and/or Sartorius

Pipettes: Eppendorf, various models

Dispensers: Brand, various models

Ultrasonic bath: Sonorex Digitec DT, Bandelin

Standard laboratory equipment: Magnetic stirrers (e.g. MIX eco 15, 2magnetic motion), Vortex apparatus (e.g. Phoenix instruments) etc.

Laboratory glass ware: Volumetric flasks, beakers, Pasteur pipettes, graduate cylinders, glass bottles etc.; different suppliers

Autosampler vials: 1.5 mL volume with PTFE sealed screw caps and septum, VWR

Centrifuge: Thermo Fisher Scientific and/or Heraeus

Chromatographic conditions

System: Waters Acquity

Software: Empower 3

Column: Acquity UPLC BEH, C18
 50 x 2.1 mm; 1.7 µm

Temperature: 60°C

Mobile Phase: A: HPLC-water + 0.2% phosphoric acid
 B: acetonitrile

Gradient Mode:	Time [min]	Solvent A [%]	Solvent B [%]
	initial	70	30
	0.33	70	30
	2.00	45	55
	2.10	5	95
	3.20	5	95
	3.30	70	30
	5.00	70	30

Total Run Time: 5 minutes

Flow Rate: 0.5 mL/min

Injection Volume: 10 µL

Detector: DA- Detector at 191 to 400 nm,
 monitoring wavelength at 209 nm

Retention Time: Pelargonic acid: 2.45 minutes

Standard Solutions used for Quantification

Stock Solution: The reference item was dissolved with acetonitrile to obtain a stock solution of approximately 1 g reference item/L. The stock solution was prepared freshly on the day of analysis.

Solvent Mixture: Acetonitrile/pure water (50/50 v/v)
Standard Solutions: Aliquots of the stock solution was diluted with pure water (diluted by factor 300 in solvent mixture) to get matrix matched standard solutions in the range from 15 to 150 mg /L for each reference item.
For evaluation of possible matrix effects solvent standards of 15 mg reference item /L and 150 mg reference item /L were prepared in solvent mixture.

Sample

Duplicate samples of approximately 10 mL from the homogenous stock solution and two samples of the matrix (deionised water) as control solution were taken from the biological ibacon study 167841087.
The concentration of the active ingredient pelargonic acid was determined in one sample of each duplicate.

Sample Preparation

Sample Preparation: After thawing an aliquot of each sample was diluted with solvent mixture to match the calibration range.
The samples were diluted, some solutions of them stirred in the process. The samples were analysed on the day of processing.
Fortification Procedure: The test item was dissolved in pure water (stirred for 30 minutes) to get fortified samples of about 45 g test item/L. These solutions were diluted with pure water to obtain fortified samples of about 22.5 g test item/L.
Afterwards fortified samples were diluted with solvent mixture to match calibration range.
Replicates: Five independent replicates per fortification level.
Two independent replicates of matrix blanks.

Results and discussions

Table A 13: Recovery results from method validation of pelargonic acid using the analytical method

Matrix	Analyte	Fortification level (g test item/L) (n = 5)	Mean recovery (%)	RSD (%)
Fortified blank GLOB2011I diluted samples.	Pelargonic acid	22.5 (n=5)	94	7
		45 (n = 5)	91	4
Overall mean value (n = 10)			93	6

Table A 14: Characteristics for the analytical method used for validation of pelargonic acid residues in fortified blank GLOB2011I diluted samples

	Pelargonic acid
Specificity	No interference above 30% of LOQ was observed in control samples at retention time of target analyte. The representative standard, blank control and fortification chromatograms show no significant interfering signals at the retention time of the analyte (Figures are provided).
Calibration (type, number of data points)	Linear

	Pelargonic acid
	$r^2 \geq 0.999$ number of data points: 8
Calibration range	15 to 150 mg pelargonic acid /L corresponding to 28% of LOQ to 140% of higher fortification level
Assessment of matrix effects is presented	Yes. No significant matrix effect ($\leq 20\%$) was observed. Matrix effect = 0% (15 mg a.s./L) and 1% (150 mg a.s./L) However, matrix-matched standards were used for determination of the active ingredients.
Limit of quantification	22.5 g a.s./L
Limit of Detection	15 mg a.s./L
Standard and Extract Stability:	The stock solution of the reference item was prepared on the day of analysis. Samples were analysed on the day of sample processing. Therefore, stability of standards and extract were not assessed.

Conclusion

The method was sufficiently validated according to SANTE/2020/12830 Rev.1. and can be used to reliably and accurately determine pelargonic acid in aqueous solutions as used in the vegetative vigour test.

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

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

No new or additional studies have been submitted

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

No new or additional studies have been submitted

A 2.2.2.3 Description of Methods for the Analysis of Soil (KCP 5.2)

No new or additional studies have been submitted

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

No new or additional studies have been submitted

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

No new or additional studies have been submitted

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

No new or additional studies have been submitted

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

No new or additional studies have been submitted