

FINAL REGISTRATION REPORT

Part B

Section 5

Analytical Methods

Detailed summary of the risk assessment

Product code: FGG01

Product name(s): Lozzare Pro, Miller Pro, Palator Pro

Chemical active substance:

Boscalid, 500 g/kg

Central Zone

Zonal Rapporteur Member State: Poland

CORE ASSESSMENT

(Article 33 application for a new product registration)

Applicant: UPL Holdings Coöperatief U.A.

Submission date: 08/05/2024

Finalisation date: 11/2024 03/2025

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Version history

When	What
08 May 2024	V0 – Version from applicant for submission to z-RMS Poland in the frame of the PPP Authorization according to Article 33 of Regulation (EC) No 1107/2009.
November 2024	zRMS assessment
March 2025	Revision after commenting stage

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5 Analytical methods

5.1 Conclusion and summary of assessment

Submitted data are sufficient for evaluation of active substance determination. There are no data gaps and conditions for authorization. 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: **None**.

Data gap: primary and ILV methods with LOQ of 0.01 mg/kg for muscle. This gap can be filled after registration (within two years).

Commodity/crop	Supported/ Not supported
Grapes	Supported
Oilseeds rape	Supported
Beans and peas	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 boscalid in plant protection product is provided as follows:

Comments of zRMS:	The analytical method (HPLC-UV) for the determination of boscalid in the formulation (Lot No. ARD/BD364/50/WG/0422/59; Content: 51.33 ± 0.04 %w/w or 513.34 ± 0.40 g/Kg) has been submitted and meets the criteria of specificity, linearity and precision according to the requirements of SANCO 3030/99 rev 5, therefore the method is acceptable.
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Reference: KCP 5.1.1/01

Report Validation of analytical method for determination of active ingredient content of Boscalid 500 g/kg WG. Chaudhari M.N., 2022, Report No. 228-2-12-31215, UPL/2022/0950, EFSA-2022-00011132

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Guideline(s): Yes
 Deviations: No
 GLP: Yes
 Acceptability: Yes

Materials and methods

The active ingredient content of Boscalid 500 g/Kg WG is determined by reversed-phase high-performance liquid chromatography on a C-18 column using acetonitrile (60%): 0.1% Orthophosphoric acid in Milli-Q water (40%) as mobile phase and UV detection at 254 nm. The test item is dispersed in a small volume of Mili-Q water before being dissolved with acetonitrile.

Instrumental Parameters:

Instrument	HPLC - UV
Column	YMC - Triart, C18 [150 mm x 4.6 mm (i.d.); 3.0 µ particle size] or equivalent
Wavelength	254 nm
Mobile Phase	Acetonitrile [60 %]: 0.1% Orthophosphoric acid in Milli Q Water [40 %], v/v
Injection Volume	10 µL
Column Temperature	40 °C
Flow Rate	1.0 mL/minute

Validation - Results and discussions

Table 5.2-1: Methods suitable for the determination of active substance Boscalid in plant protection product FGG01

	Boscalid			
Author(s), year	Chaudhari M.N., 2022			
Principle of method	HPLC-UV			
	Parameters			Results
Linearity (linear between mg/L / % range of the declared content) (correlation coefficient, expressed as r)				For Repeatability-1 and Repeatability-2
	Concentration Range (mg/L)			155.10 to 371.80
	Concentration Range (% w/w)			31.10 to 74.54
	Intercept (a)			243090.70
	Slope (b)			25899.60
	Correlation Coefficient			0.999
Precision – Repeatability Mean n = 5	Repeatability-1	Mean Content	% w/w ± SD	51.34 ± 0.04
			g/kg ± SD	513.36 ± 0.38
		(% RSD)		0.08

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	Boscalid					
(%RSD)		Acceptable % RSD (Extended Horwitz)		1.48		
		Horrat Value (Hr)		0.05		
		Acceptable Horrat Value (Hr)		≤ 1		
	Repeatability-2	Mean Content	% w/w ± SD		51.33 ± 0.05	
			g/Kg ± SD		513.32 ± 0.46	
		(% RSD)		0.1		
		Acceptable % RSD (Extended Horwitz)		1.48		
		Horrat Value (Hr)		0.07		
		Acceptable Horrat Value (Hr)		≤ 1		
		Intermediate Precision	Overall Mean Content	% w/w ± SD		51.33 ± 0.04
	g/Kg ± SD			513.34 ± 0.40		
	(% RSD)		0.08			
	Acceptable % RSD (Horwitz)		2.21			
Accuracy n = 9 (% Recovery)	Level (mg/L) / Mean % Recovery			Overall Mean % Recovery	Acceptable Limit %	
	I (203.37)	II (255.30)	III (306.03)			
	99.73	100.36	100.61	100.23	98 - 102	
Interference/ Specificity	No interference					
Comment	The analytical method was accurately validated covering specificity, linearity, precisions and recovery (accuracy) according with guideline SANCO/3030/99 re.v5					

Conclusion

From the results of the analytical method validation, it is concluded that the analytical method is specific, precise, and accurate for the analysis of Boscalid 500 g/Kg WG. Results of the validation criteria are within the specified limits of SANCO/3030/99 rev. 5, OCSPP 830.1800, and ABNT NBR 14029 guidelines. The analytical method is suitable for the determination of boscalid in plant protection product FGG01.

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

According to regulation 540/2011, there are no relevant impurities in technical boscalid.

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

Under current EU legislation, analytical methods for the determination of formulants are not required.

5.2.1.4 Applicability of existing CIPAC methods (KCP 5.1.1)

The CIPAC analytical method 673 is available for the determination of boscalid. According to CIPAC 673, boscalid is determined by reversed phase high performance liquid chromatography using UV detection at 260 nm and external standardisation. The method is usable for TC, WG, SE, SC-formulations.

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

Component of residue definition: boscalid				
Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing / EU agreed
Tomato, lemon, grain oilseed rape (Residues)	Primary (DFG S19)	0.01 mg/kg 0.02 mg/kg	GC-MS	Weeren R.D. and Pelz S., 1999 / EU agreed (DAR 2002) 1999/11461
	Confirmatory (if required)	-		
Apple, sour cherry, grapes, strawberry, carrot, onion, tomato, broccoli, cabbage, leek, dwarf beans, oilseed rape (Residues)	ILV of DFG S19	0.05 mg/kg	LC-MS-MS	Reichert N., 2001/ EU agreed (DAR 2002) 2000/1014886
	Confirmatory (if required)	-		
White cabbage, lettuce oilseed rape hops (Residues)	Primary (DFG S19)	0.01 mg/kg	GC-MS	Class T., 2001 / EU agreed (DAR 2002) 2000/1017227
	Confirmatory (if required)	-		
Apple, sour cherry, grapes, strawberry, carrot, onion, tomato, broccoli, cabbage, leek, dwarf beans, oilseed rape (Residues)	Primary (445/0)	0.05 mg/kg	LC-MS/MS	Funk H. und Mackenroth Ch., 2000 / EU agreed (DAR 2002) 2000/1012404
	Confirmatory (if required)	-		

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Component of residue definition: boscalid				
Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing / EU agreed
Wheat grains, wheat straw, lettuce, whole orange, oilseed rape seeds (Residues)	Primary	0.01 mg/kg	LC-MS/MS	Rastogi P.K., 2022 / New study not previously reviewed S22-06799 Used in studies: S22-01574 and S22-01575 (please refer to dRR part B7, KCP 7.2.3.2/02 and KCP 7.2.3.3/01)
	Confirmatory (if required)	Not required (two mass transitions)		
Milk, muscle, fat, kidney, liver (cow) egg (hen) (Residues)	Primary (DFG S19)	0.01 mg/kg (milk) and 0.025 mg/kg (others)	GC-ECD and GC-MS	Class T., 2001 / EU agreed (DAR 2002) 2000/1017227
	Confirmatory (if required)	-		
Milk, liver (cow) (Residues)	Primary (DFG S19)	0.01 mg/kg (milk) and 0.025 mg/kg (others)	GC-ECD	Class, T., 2001 / EU agreed (DAR 2002) 2000/1017227
Milk, cream, egg, muscle, liver, kidney, fat (Residues)	Primary (471/0)	0.01 mg/kg (milk, cream, eggs) and 0.025 mg/kg (others)	LC-MS/MS	Kampke-Thiel, K., 2001 / EU agreed (DAR 2002) 2000/1017226
Milk, liver (cow) (Residues)	Primary (476/0)	0.01 mg/kg (milk) and 0.05 mg/kg (liver)	GC-MS	Fabian, E., 2001 / EU agreed (DAR 2002) 2000/1017224
Water (Environmental fate)	Primary	0.05 mg/kg	GC-MS	Keller, W., 1998 / EU agreed (DAR 2002) 1998/10922
	Primary	0.05 mg/kg	GC-MS	Grote, C., 2003 / EU agreed (Addendum 2006) 2003/1000976
	Primary	0.05 mg/kg	GC-MS	Grote, Ch., 2001/ EU agreed (DAR 2002) 2001/1008955
	Primary	0.05 mg/kg	GC-MS	Grote, C., 2003 / EU agreed (Addendum 2006) 2003/1000975
	Confirmatory (if required)	-		
Soil (Environmental fate)	Primary	0.01 mg/kg	GC-MS	Keller, W., 1998 / EU agreed (DAR 2002) 1998/11314
	Primary	0.01 mg/kg	GC-MS	Grote, C. 2003 / EU agreed

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Component of residue definition: boscalid				
Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing / EU agreed
				(Addendum 2006) 2003/1000977
	Confirmatory (if required)	-		
Air	Primary	1.5 µg/m ³	GC-MS	Zangmeister, W., 2000 / EU agreed (DAR 2002) 2000/1014992
	Confirmatory (if required)	-		
Matrix (Ecotoxicology)	Primary	4.39 mg/L (Reconstituted water) 0.31 mg/L (1X AAP)	HPLC-UV	Pandey, P.K., 2023 / New study not previously reviewed 228-2-13-31234 Used in studies: 502-3-07-31231 – Daphnia and 501-3-07-31230 - algae
	Confirmatory (if required)	-		
Matrix (Ecotoxicology)	Primary	125 g test item/L corresponding to 6.4 mg Boscalid/L after dilution by factor 10000 (Tap water with 0.1 % v/v Triton X-100) 17.5 g test item/L corresponding to 4.5 mg Boscalid/L after dilution by factor 2000 (50 % w/v sucrose solution)	HPLC-UV	Knautz, T., Kowalczyk, F. 2022a / New study not previously reviewed UPL/2022/2874 165091105 (Analytical phase)
	Confirmatory (if required)	-		
Feeding solution (Ecotoxicology)	Primary	1.0 g test item/L corresponding to 5.1 mg Boscalid/L after dilution by factor 100	HPLC-UV	Knautz, T., Kowalczyk, F. 2022b / New study not previously reviewed UPL/2022/2873 165091136 (Analytical phase)

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Component of residue definition: boscalid				
Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing / EU agreed
	Confirmatory (if required)	-		
Aqueous solution (Ecotoxicology)	Primary	0.21 g Boscalid/L	HPLC-UV	Colli, M., 2022 / New study not previously reviewed UPL/2022/2796 BT115/22 (Analytical phase)
	Confirmatory (if required)	-		
Dosing solution (Ecotoxicology)	Primary	12.5 g test item/L corresponding to 12.9 mg Boscalid /L after dilution by factor 500	HPLC-UV	Stürtz, S., Horstmann, C., 2022 a / New study not previously reviewed UPL/2022/0597 165091086 (Analytical phase)
	Confirmatory (if required)	-		
Dosing solution (Ecotoxicology)	Primary	12.5 g test item/L corresponding to 12.9 mg Boscalid /L after dilution by factor 500	HPLC-UV	Stürtz, S., Horstmann, C., 2022 b / New study not previously reviewed UPL/2022/0598 165091087 (Analytical phase)
	Confirmatory (if required)	-		

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

Component of residue definition: M510F01				
Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing / EU agreed
milk, muscle, fat, kidney, liver (cow) egg (hen) (Residues)	Primary (DFG S19)	0.01 mg/kg (milk) and 0.025 mg/kg (others)	GC-ECD and GC- MS	Class, T., 2001 / EU agreed (DAR 2002) 2000/1017227

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Component of residue definition: M510F01				
Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing / EU agreed
	Confirmatory (if required)	-		
milk, liver (cow) (Residues)	Primary (DFG S19)	0.01 mg/kg (milk) and 0.025 mg/kg (others)	GC-ECD	Kampke-Thiel, K., 2001 / EU agreed (DAR 2002) 2000/1017226
milk, cream, egg, muscle, liver, kidney, fat (Residues)	Primary (471/0)	0.01 mg/kg (milk, cream, eggs) and 0.025 mg/kg (others)	LC-MS/MS	Grosshans, F., 2001/ EU agreed (DAR 2002) 2000/1017223
milk, liver (cow) (Residues)	Primary (476/0)	0.01 mg/kg (milk) and 0.05 mg/kg (liver)	GC-MS	Fabian E., 2001 / EU agreed (DAR 2002) 2000/1017224

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)

Please refer to analytical methods provided in point 5.2.1.

5.3.2 Description of analytical methods for the determination of Boscalid (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 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	Boscalid	1.50 mg/kg (pears) 3.00 mg/kg (fresh beans and peas without pods, plums) 5.00 mg/kg (fresh beans and peas with pods, stone fruits)	Reg. (EU) 2022/1324

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Matrix	Residue definition	MRL / limit	Reference for MRL/level Remarks
Plant, high acid content		5.00 mg/kg (table and wine grapes, kiwi)	Reg. (EU) 2022/1324
Plant, high protein/high starch content (dry commodities)		0.15 mg/kg	Reg. (EU) 2022/1324
Plant, high oil content		1 mg/kg (flax, oilseed rape)	Reg. (EU) 2022/1324
Plant, difficult matrices (hops, spices, tea)		0.05 mg/kg	Reg. (EU) 2022/1324
Muscle	Boscalid in muscle, fat, milk and eggs and the sum of boscalid and its hydroxy metabolite M510F01 (free and conjugated), expressed as boscalid, in liver and kidney	0.01 mg/kg	Reg. (EU) 2022/1324
Milk		0.02 mg/kg	Reg. (EU) 2022/1324
Eggs		0.01 mg/kg	Reg. (EU) 2022/1324
Fat		0.01 mg/kg	Reg. (EU) 2022/1324
Liver, kidney		0.01 mg/kg	Reg. (EU) 2022/1324
Honey	Boscalid	0.15	Reg. (EU) 2022/1324
Soil (Ecotoxicology)	Boscalid	0.05 mg/kg	Common limit
Drinking water (Human toxicology)	Boscalid and metabolite M510F049	0.1 µg/L	General limit for drinking water
Surface water (Ecotoxicology)	Boscalid	NOEC: 0.125 µg/L	The value refers to study on fish. SANCO/3919 /2007-rev. 5
Air	Boscalid	30 µg/m ³	AOEL sys: 0.1 mg/kg bw/d SANCO/3919 /2007-rev. 5
Tissue (meat or liver)	Not required as the active substance is not toxic or very toxic	0.01 (※) animal mg/kg	Not classified as T / T+ SANTE/2020/12830, Rev.2 14. February 2023
Body fluids	Boscalid	Not required 0.01 mg/L	Not classified as T / T+ SANTE/2020/12830, Rev.2 14. February 2023

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 Boscalid in plant matrices is given in the following tables. For the detailed evaluation of new/ additional studies it is referred to Appendix 2.

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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: Boscalid				
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	GC-MS	Weeren and Pelz, 1999 / EU agreed / DAR 2002 DocID 1999/11461
	ILV	0.01 mg/kg	GC-MS	Reichert, 2001 / EU agreed / DAR 2002 DocID 2000/1014886
	Confirmatory (if required)	Not required as ILV is highly selective		
	Primary	0.05 mg/kg	HPLC-MS/MS	Funk and Mackenroth, 2001/ EU agreed / DAR 2002 DocID 2000/1012404
	ILV	As a vast number of validated enforcement methods using QuEChERS approach is available from the EURL (European Reference Laboratories for Residues of Pesticides) Data Pool, no additional ILV (independent laboratory validation) is required.		
	Confirmatory (if required)	Not necessary (two mass transitions)		
High acid content	Primary	0.01 mg/kg	GC-MS	Weeren and Pelz, 1999 / EU agreed / DAR 2002 DocID 1999/11461
	ILV	0.01 mg/kg	GC-MS	Reichert, 2001 / EU agreed / DAR 2002 DocID 2001/1014886
	Confirmatory (if required)	Not required as ILV is highly selective		
	Primary	0.05 mg/kg	HPLC-MS/MS	Funk and Mackenroth, 2001/ EU agreed / DAR 2002 DocID 2000/1012404
	ILV	As a vast number of validated enforcement methods using QuEChERS approach is available from the EURL (European Reference Laboratories for Residues of Pesticides) Data Pool, no additional ILV (independent laboratory validation) is required.		
	Confirmatory (if required)	not necessary (two mass transitions)		
High oil content	Primary	0.02 mg/kg (rapeseed oil)	GC-MS	Weeren and Pelz, 1999 / EU agreed / DAR 2002 DocID 1999/11461
	ILV	0.02 mg/kg	GC-MS	Reichert, 2001 / EU agreed / DAR 2002 DocID 2001/1014886

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Component of residue definition: Boscalid				
Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing / EU agreed
	Confirmatory (if required)	Not necessary as ILV is highly selective		
	Primary	0.05 mg/kg	HPLC-MS/MS	Funk and Mackenroth, 2001/ EU agreed / DAR 2002 DocID 2000/1012404
	ILV	As a vast number of validated enforcement methods using QuECHERS approach is available from the EURL (European Reference Laboratories for Residues of Pesticides) Data Pool, no additional ILV (independent laboratory validation) is required.		
	Confirmatory (if required)	Not necessary (two mass transitions)		
High protein/high starch content (dry)	Primary	0.01 mg/kg	GC-MS	Weeren and Pelz 1999 / EU agreed / DAR 2002 DocID 1999/11461
	ILV	Not necessary as ILV is performed in high water and high oil content		
	Confirmatory (if required)	Not necessary as the method is highly selective		
Difficult (if required, depends on intended use)	Primary	0.05 mg/kg (hops, recovery = 63 %)	HPLC-MS/MS	Reichert, 2001 / EU agreed / DAR 2002 DocID 2001/1014886
	Not required as hops are not included in the intended uses			

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:	Investigations on the extractability of 14C-BAS 510 F residues from plant matrices; Bross, M., 2001 Study code 73479 2001/1001739 GLP Unpublished MET2001-268 which is already peer-reviewed in the DAR for the evaluation of boscalid (DAR, 2002).
Comment on extraction efficiency:	As the extraction procedure used in the residue analytical method 445/0 and the multi residue method S19 slightly deviates from those used in the metabolism studies, ¹⁴ C nicobifen treated plant material was extracted according to these methods and the results thereof were compared. Purpose of this study is to verify the extraction efficiency of the “cold methods” in extracting the total

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	Method for products of plant origin
	radioactive residue (TRR). The extraction efficiency is proven sufficiently for commodities with high water content, with high oil content, for dry and acidic crops.

For the detailed evaluation of (additional) studies on extraction efficiency, it is referred to 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 Boscalid in animal matrices is given in the following tables. For the detailed evaluation of new/ additional studies it is referred to Appendix 2.

zRMS: the LOQ of the method of Class, 2000 for eggs, and muscle are not sufficient for currently stated MRLs.

Data gap: primary and ILV methods with LOQ of 0.01 mg/kg for muscle.

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

Component of residue definition: Boscalid and metabolite M510F01				
Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing / EU agreed
Milk	Primary	0.01mg/kg	GC-MS	Class, T., 2001 / EU agreed / DAR 2002 DFG S19 method 2000/1017227
	ILV	0.01 mg/kg	GC-MS	Kampke-Thiel, 2001 / EU agreed / DAR 2002 DFG S19 method 2000/1017226
	Confirmatory (if required)	Not necessary as the ILV is highly selective		
	Primary	0.01mg/kg	HPLC-MS/MS	Grosshans, F, 2001 / EU agreed / DAR 2002 BASF-method 471/0 2000/1017223
	ILV	As a vast number of validated enforcement methods using QuEChERS approach is available from the EURL (European Reference Laboratories for Residues of Pesticides) Data Pool, no additional ILV (independent laboratory validation) is required.		
	Confirmatory (if required)	Not necessary (two mass transitions)		
	Primary (only for bound metabolite M510F53)	0.01 mg/kg	GC-MS	Fabian, E. / 2001 / EU agreed / DAR 2002 BASF-method 476

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Component of residue definition: Boscalid and metabolite M510F01				
Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing / EU agreed
				2000/1017224
Eggs	Primary	0.025 mg/kg	GC-MS	Class, T., 2001 / EU agreed / DAR 2002 DFG S19 method 2000/1017227
	ILV	Not necessary as ILV is provided for milk and liver		
	Confirmatory (if required)	Not necessary as the method is highly selective		
	Primary	0.01 mg/kg	HPLC-MS/MS	Grosshans, F, 2001 / EU agreed / DAR 2002 BASF-method 471/0 2000/1017223
	ILV	As a vast number of validated enforcement methods using QuEChERS approach is available from the EURL (European Reference Laboratories for Residues of Pesticides) Data Pool, no additional ILV (independent laboratory validation) is required.		
	Confirmatory (if required)	Not necessary (two mass transitions)		
Muscle	Primary	0.025 mg/kg	GC-MS	Class, T., 2001 / EU agreed / DAR 2002 DFG S19 method 2000/1017227
	ILV	Not necessary as ILV is provided for milk and liver		
	Confirmatory (if required)	Not necessary as the method is highly selective		
	Primary	0.025 mg/kg	HPLC-MS/MS	Grosshans, F, 2001 / EU agreed / DAR 2002 BASF-method 471/0 2000/1017223
	ILV	As a vast number of validated enforcement methods using QuEChERS approach is available from the EURL (European Reference Laboratories for Residues of Pesticides) Data Pool, no additional ILV (independent laboratory validation) is required.		
	Confirmatory (if required)	Not necessary (two mass transitions)		
Fat	Primary	0.025 mg/kg	GC-MS	Class, T., 2001 / EU agreed / DAR 2002 DFG S19 method 2000/1017227
	ILV	Not necessary as ILV is provided for milk and liver		
	Confirmatory (if required)	Not necessary as the method is highly selective		

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Component of residue definition: Boscalid and metabolite M510F01				
Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing / EU agreed
	Primary	0.025 mg/kg	HPLC-MS/MS	Grosshans, F, 2001 / EU agreed / DAR 2002 BASF-method 471/0 2000/1017223
	ILV	As a vast number of validated enforcement methods using QuEChERS approach is available from the EURL (European Reference Laboratories for Residues of Pesticides) Data Pool, no additional ILV (independent laboratory validation) is required.		
	Confirmatory (if required)	Not necessary (two mass transitions)		
Kidney, liver	Primary	0.025 mg/kg	GC-MS	Class, T., 2001 / EU agreed / DAR 2002 DFG S19 method 2000/1017227
	ILV	0.025 mg/kg	GC-MS	Kampke-Thiel, 2001 / EU agreed / DAR 2002 DFG S19 method 2000/1017226
	Confirmatory (if required)	Not necessary as the ILV is highly selective		
	Primary	0.025 mg/kg	HPLC-MS/MS	Grosshans, F, 2001 / EU agreed / DAR 2002 BASF-method 471/0 2000/1017223
	ILV	As a vast number of validated enforcement methods using QuEChERS approach is available from the EURL (European Reference Laboratories for Residues of Pesticides) Data Pool, no additional ILV (independent laboratory validation) is required.		
	Confirmatory (if required)	Not necessary (two mass transitions)		
	Primary (only for bound metabolite M510F53)	0.05 mg/kg	GC-MS	Fabian, E., 2001 / EU agreed / DAR 2002 BASF-method 476 2000/1017224
Honey	Primary	0.01 mg/kg	GC-MS/MS	Sahvorost, N., 2022 / New study not previously reviewed UPL/2022/0354 S22-00776
	ILV	0.01 mg/kg	GC-MS/MS	Rastogi, T., 2022 / New study not previously reviewed UPL/2022/0845 S22-00783

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Component of residue definition: Boscalid and metabolite M510F01				
Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing / EU agreed
	Confirmatory (if required)	Not necessary (two mass transitions)		

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:	Extraction efficiency has been assessed during the Annex I inclusion process (DAR 2002). The extraction solvent used in the residue analytical method 471/0 (methanol) as well as in the multimethod approach DFG S19 removed comparable amounts of residues than the metabolism extraction scheme.

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

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 boscalid 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: Boscalid			
Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing / EU agreed
Primary	0.01 mg/kg	GC-MS	Keller, W., 1998a / EU agreed / DAR 2002 BASF-method 408/1 1998/11314 Grote C., 2003 / EU agreed / ADD 2006 2003/1000977
Confirmatory	Not necessary as the method is highly selective		

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 boscalid 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.

zRMS:

data gap: ILV method for drinking water.

This data gap is anticipated to be addressed at active substance level in context with the renewal.

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

Component of residue definition: Boscalid				
Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing / EU agreed
Drinking water	Primary	0.05 µg/L	GC-MS	Grothe, Ch., 2001 / EU agreed / DAR 2002 BASF-method 411/0 2001/1008955 Grote C., 2003 / EU agreed / ADD 2006 2003/1000975
	ILV	0.1 µg/L	GC-MS/MS	Kaiser, M., 2023 / New study not previously reviewed UPL/2022/21136 S22-01028
	Confirmatory	Not required (two mass transitions)		
Surface water	Primary	0.05 µg/L	GC-MS	Keller, W., 1998 / EU agreed / DAR 2002 BASF-method 411 1998/10922 Grote C., 2003 / EU agreed / ADD 2006 2003/1000976
	Confirmatory	Not necessary as the method is highly selective		

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 boscalid in air is given in the following tables. For the detailed evaluation of new/ additional studies please refer to Appendix 2.

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

Component of residue definition: boscalid			
Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing / EU agreed
Primary	1.5 µg/m ³	GC-MS	Zangmeister, W., 2000 / EU agreed (DAR 2002) 2000/1014992
Confirmatory	Not necessary as the method is highly selective		

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 boscalid 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: boscalid and metabolite M510F01			
Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing / EU agreed
Primary	0.01 mg/kg	An entry is missing here LC-MS/MS LOQ: 0.01 mg/L (blood, urine)	Rastogi, T., 2022 / New study not previously reviewed UPL/2022/2054 S22-00982 Appendix 2
Confirmatory	Not required (two mass transitions)		

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

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Appendix 1 Lists of data considered in support of the evaluation

The following lists should include all product data considered in support of the evaluation, even if they have been evaluated previously, e.g. in the EU peer review of the active substance(s), and thus are not summarised in this document in detail. New data evaluated for the active substance(s) should be included as well.

Please sort by data points and within one data point by names of authors.

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 5.1.1/01	Chaudhari M.N	2022	Validation of analytical method for determination of active ingredient content of Boscalid 500 g/kg WG UPL/2022/0950 Jai Research Foundation 228-2-12-31215 GLP Unpublished	No	UPL
KCP 5.1.2/01	Rastogi T.	2022	Validation of the Analytical Method for Determination of Boscalid in Different Matrices of Plant Origin. Study UPL/2022/2099 Eurofins Agroscience Services, EAG Laboratories GmbH S22-06799 GLP Unpublished	No	UPL

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Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 5.1.2/02	Rastogi T.	2023	Cross-Validation - Comparing Amounts of Boscalid extracted from Samples of Plant Origin with incurred Residues using three different Solvent Systems. UPL/2023/1062 Eurofins Agroscience Services, EAG Laboratories GmbH S22-00983 GLP Unpublished	No	UPL
KCP 5.1.2/03	Pandey P.K.	2023	Validation of Analytical Method for Determination of Active Ingredient Concentration and Stability of Boscalid in Matrix, following the Application of Boscalid 500 g/kg WG UPL/2022/2648 Jai Research Foundation 228-2-13-31234 GLP Unpublished	N	UPL
KCP 5.1.2/04	Knautz T. Kowalczyk F.	2022a	Boscalid 500 WG: Acute Contact and Oral Toxicity to Bumblebees (Bombus terrestris L.) in the Laboratory UPL/2022/2874 ibacon GmbH 165091105 GLP Unpublished	N	UPL
KCP 5.1.2/05	Knautz T. Kowalczyk F.	2022b	Boscalid 500 WG: Chronic Oral Toxicity Test on the Honey Bee (Apis mellifera L.) in the Laboratory UPL/2022/2873 ibacon GmbH 165091136, GLP Unpublished	N	UPL

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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 5.1.2/06	Colli M.	2022	Effects of BOSCALID 500 WG (FGG01) on honeybees (Apis mellifera L.) 22-day larval toxicity test with repeated exposure. UPL/2022/2796 BioTecnologie BT S.r.l. BT115/22 GLP Unpublished	N	UPL
KCP 5.1.2/07	Stürtz S., Horstmann C.,	2022 a	Boscalid 500 WG: Effects on Terrestrial (Non-Target) Plants: Seedling Emergence and Seedling Growth Test UPL/2022/0597 ibacon GmbH 165091086 GLP Unpublished	N	UPL
KCP 5.1.2/08	Stürtz S., Horstmann C.	2022 b	Boscalid 500 WG: Effects on Terrestrial (Non-Target) Plants: Vegetative Vigour Test UPL/2022/0598 ibacon GmbH 165091087 GLP Unpublished	N	UPL
KCP 5.2/01	Sahvorost N.	2022	Validation of the Analytical Method for the Determination of Residues of Boscalid in Honey, Report Amendment 1, UPL/2022/0354 Eurofins Agroscience Services EcoChem GmbH S22-00776 GLP Unpublished	N	UPL

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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 5.2/02	Rastogi T.	2022	Independent Laboratory Validation of the Analytical method for Determination of Residues of Boscalid in Honey UPL/2022/0845 Eurofins Agroscience Services EcoChem GmbH S22-00783 GLP Unpublished	N	UPL
KCP 5.2/03	Kaiser M.	2023	Independent Laboratory Validation of an Analytical Method for Determination of Boscalid and its Metabolites M510F47, M510F49 and M510F64 in Ground and Drinking Water, UPL/2022/21136, Eurofins Agroscience Services EcoChem GmbH S22-01028 GLP Unpublished	N	UPL
KCP 5.2/04	Rastogi T.	2022	Development and Validation of an Multi-Residue Method QuEChERS for the Determination of Boscalid and its Metabolite M510F01 in Body Fluids UPL/2022/2054 Eurofins Agroscience Services, EAG Laboratories GmbH S22-00982 GLP Unpublished	N	UPL

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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
CP 5.1.2	Weeren, R.D., Pelz, S.	1999	Validation of DFG method S19 for the determination of BAS 510 F in various plant materials Az. M8020/99 1999/11461 GLP Unpublished	N	BAS
CP 5.1.2	Reichert, N.	2001	Independent laboratory validation of a method of analysis for the determination of BAS 510 F in white cabbage, rape (seed), hop, and lettuce IF-100/35725-00 2000/1014886 GLP Unpublished	N	BAS
CP 5.1.2	Class, T.	2001	Assessment and validation of the adapted multi-residue method DFG S19 for the determination of BAS 510 F and its metabolite M510F01 in animal matrices Report no. P/B 453 G. 2000/1017227 GLP, Unpublished	N	BAS
CP 5.1.2	Funk, H., Mackenroth, Ch.	2001	Validation of BASF method no. 445/0: determination of BAS 510 F in plant matrices; study code 41840. 2000/1012404 GLP Unpublished	N	BAS
CP 5.1.2	Fabian, E.	2001	The validation of BASF method 476/0: the determination of BAS 510 F residues (as M510F53) in liver and milk by microwave treatment Study code 96997. 2000/1017224	N	BAS

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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
			GLP Unpublished		
CP 5.1.2	Keller, W.	1998b	Validation of analytical method no. 411, determination of BAS 510 F ai residues in water; study no. 41877. 1998/10922 GLP, Unpublished	N	BAS
CP 5.1.2	Grote, C	2003b	Report Amendment No.1 to Validation of analytical method No. 411. Determination of BAS 510 F ai residues in water; BASF Doc ID 2003/1000976 unpublished	N	BAS
CP 5.1.2	Grote, Ch.	2001	Validation of analytical method no. 411/0, GC/MS determination of BAS 510 F ai residues in surface water; study code 110241. 2001/1008955 GLP, unpublished	N	BAS
CP 5.1.2	Grote, C	2003	Report Amendment No.1 to Validation of analytical method No. 411/0. GC/MS determination of BAS 510 F ai residues in surface water BASF Doc ID 2003/1000975 unpublished	N	BAS
CP 5.1.2	Keller, W.	1998	Validation of analytical method no. 408/1, GCMS determination of BAS 510 F active ingredient residues in soil and sediment after methanol extraction; study code 48541. 1998/11314 GLP, Unpublished	N	BAS

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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
CP 5.1.2	Grote, C.	2003	Report Amendment No.1 to Validation of analytical method No. 408/1. GC-MS determination of BAS 510 F active ingredient residues in soil and sediment after methanol extraction; BASF Doc ID 2003/1000977 Unpublished	N	BAS
CP 5.1.2	Zangmeister, W.	2000	Validation of analytical method 460, determination of BAS 510 F (Reg.no. 300355) in air by GC-MS; study code 41886. BASF Doc ID 2000/1014992 GLP Unpublished	N	BAS
CP 5.1.2	Kampke-Thiel, K.	2001	Independent laboratory validation of the adapted multi-residue method DFG S19 for the determination of BAS 510 F and its metabolite M510F01 in animal matrices PTRL Europe Study No. P453G. 2000/1017226 GLP Unpublished	N	BAS
CP 5.1.2	Grosshans, F.	2001	The validation of BASF method 471/0: The determination of BAS 510F and the metabolite M510F01 in animal matrices Study code 42392. 2000/1017223 GLP Unpublished	N	BAS
CP 5.1.2	Bross, M.	2001	Investigations on the extractability of 14C-BAS 510 F residues from plant matrices; study code 73479. 2001/1001739 GLP Unpublished	N	BAS

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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

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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 Boscalid

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

A 2.1.1.1 Description of analytical methods for the determination of residues in plant matrices (KCP 5.1)

A 2.1.1.1.1 Analytical method 1

A 2.1.1.1.1.1 Method validation

Comments of zRMS:	Study is accepted
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Reference:	KCP 5.1.2/01
Report	Validation of the Analytical Method for Determination of Boscalid in Different Matrices of Plant Origin, Rastogi, T., 2022, Study Number: S22-06799, Sponsor Study Number: UPL/2022/2099
Guideline(s):	Yes, SANTE/2020/12830, Rev.1 (February 24, 2021)
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

After preparation of the sample material for analysis (the sample material was thoroughly homogenised in a knife mill using dry ice), wheat grain, lettuce, whole orange and oilseed rape seed matrices were stored at $\leq -18^{\circ}\text{C}$ (frozen condition) in the dark whereas wheat straw matrix was stored at room temperature in the dark before weighing, fortification and extraction.

Samples of investigated matrices were extracted with the mixture of methanol/water/2N HCl (70/25/5, v/v/v). The samples were shaken using horizontal shaker. An aliquot of the raw extract was then transferred into the centrifuge tubes and centrifuged for about 5 minutes. The samples were diluted (DF2) in methanol/water (1/1, v/v) prior to the LC-MS/MS analysis for the determination of boscalid.

Results and discussions

The objective of the study was to develop and validate the analytical method for the determination of boscalid in/on different matrices of plant origin in accordance to guidance document SANTE/2020/12830, rev.1 for risk assessment.

The limit of quantification was 0.01 mg/kg for all matrices.

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

Matrix	Analyte	Fortification level (mg/kg) (n = 5)	Mean recovery (%)	RSD (%)	Comments
Wheat grains	Boscalid	0.01	104	2.7	Range: 99.6 - 107
		0.1	99.8	4.3	Range: 95.6 - 105
Wheat straw	Boscalid	0.01	88.5	5.1	Range: 80.8 - 92.8
		0.1	86.6	1.0	Range: 85.4 - 87.6
Lettuce	Boscalid	0.01	106	4.3	Range: 102 - 114
		0.1	94.9	11	Range: 85.6 - 107
Whole orange	Boscalid	0.01	105	2.0	Range: 102 - 108
		0.1	92.5	3.3	Range: 88.0 - 95.6
Oilseed rape seed	Boscalid	0.01	94.7	6.6	Range: 86.4 - 101
		0.1	89.5	3.3	Range: 85.6 - 93.8

Table A 2: Characteristics for the analytical method used for validation of boscalid residues in plants

	Boscalid
Specificity	Demonstrated by validation of two (2) mass transitions. Analyte levels or chromatographic interferences of unknown compounds in a reagent blank and control sample extracts were below what would correspond to an analyte level of 30% of the LOQ.
Calibration (type, number of data points)	Linear regression with 1/x weighting. Regression residuals randomly distributed Correlation coefficients (R) ≥ 0.99
Calibration range	Matrix-matched calibration standards. A minimum of seven (7) concentration levels. Single determination. Injection of standard solutions spread over the whole acquisition batch. Concentration range: 0.075 ng/mL to 7.5 ng/mL. Corresponding mass fraction range: 0.003 mg/kg to 0.30 mg/kg. Coverage: 30% of the LOQ to at least + 20% of the highest analyte concentration level detected in a diluted sample extract. The validated range does not exceed two (2) orders of magnitude.
Assessment of matrix effects is presented	Yes Insignificant (< 20%) for all matrices except wheat grain and wheat straw. Matrix-matched standards were used for quantification throughout the study.
Limit of determination/quantification	LOD: 0.003 mg/kg LOQ: 0.01 mg/kg
Stability of Working Solution	The stability of the working solution (i.e. stock, fortification and intermediate calibration solutions) of boscalid in

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	Boscalid
Specificity	Demonstrated by validation of two (2) mass transitions. Analyte levels or chromatographic interferences of unknown compounds in a reagent blank and control sample extracts were below what would correspond to an analyte level of 30% of the LOQ.
	methanol was demonstrated for 16 days when stored at 1°C to 10°C in the dark in the study S22-00980.
Stability of Final Extracts	Recoveries within 70% - 120% in all matrix extracts for at least 11 days when stored at typically 1 °C to 10 °C in the dark.

Conclusion

The method was successfully validated for the determination of boscalid in/on different matrices of plant origin (dry, high water, high acid and high oil commodities) from the tested LOQ of 0.01 mg/kg according to the guidance document SANTE/2020/12830, rev. 1 for risk assessment and meets the requirements of SANTE/2020/12830, rev. 2.

A 2.1.1.1.1.2 Confirmatory method

No confirmatory method is required (two mass transitions).

A 2.1.1.1.1.3 Extraction efficiency

Extraction efficiency of the solvent systems as used in the data generation method S22-06799 is demonstrated in the below study.

Reference:	KCP 5.1.2/02
Report	Cross-Validation - Comparing Amounts of Boscalid extracted from Samples of Plant Origin with incurred Residues using three different Solvent Systems, Rastogi, T., 2023, Study Number: S22-00983, Sponsor Study Number: UPL/2023/1062
Guideline(s):	Yes, SANTE/2017/10632, rev. 4 and SANTE/2020/12830, Rev.2
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Study Objective

The objective of the study was to compare residue amounts of boscalid extracted from samples of wheat whole plant (high water content matrix), wheat grains (dry matrix) and oilseed rape seeds (high oil content matrix) with incurred residues when extracting with different solvent systems as used in pre and post registration methods and when extracting with different solvent systems as were used in metabolism studies in accordance to the technical guideline on the evaluation of extraction efficiency of residue analytical

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methods, SANTE/2017/10632, rev. 4. All methods were validated in accordance with SANTE/2020/12830, rev. 2.

Materials and methods

The samples analysed in the current study were derived from the raw agricultural commodity of matrices obtained in the corresponding residues studies/analytical phases (wheat whole plant, wheat grains and oilseed rape seeds). Samples from those matrices were used for the extraction with the different solvent systems.

The samples were homogenised with the respective analytical phases.

Table A 3: Extraction methods used for the cross validation

Method 1: Multi-residue method	
Reference(s)	S22-00980
Solvent System for Extraction	Addition of water (if necessary) and extraction with acetonitrile
Validation Status	All matrix categories occurring in this study have previously been validated at the test facility as can be taken from the reference given above
Method 2: Data generation / risk assessment method	
Reference(s)	S22-06799 [KCP 5.1.2/01]
Solvent System for Extraction	Mixture of methanol, water and hydrochloric acid
Validation Status	All matrix categories occurring in this study have previously been validated at the test facility as can be taken from the reference given above.
Method 3: Metabolism method	
Reference(s)	Metabolism study [Vol 3-B7, RAR 2018]
Solvent System for Extraction	Extraction with methanol
Validation Status	A full validation was part of this study

Results and discussions

Table A 4: Summary evaluation of extraction efficiency of residue analytical methods

Item	Activity, Result, Assessment
Analyte	Boscalid
Matrices	Wheat grain (dry commodity), wheat whole plant (high water content commodity), oilseed rape seeds (high oil content matrix)
Method References	Methods developed in Study S22-00980 and S22-06799 [KCP 5.1.2/01], Metabolism Method [Vol 3-B7, RAR 2018]
LOQ	0.01 mg/kg (lowest validated fortification level) for all matrices
LOD	≤30 % of the LOQ
Principle of the Analytical Procedure	Homogenisation: Dry ice, knife mill or cutter Method S22-00980 (Method 1): Extraction: Acetonitrile and addition of water (if needed). Ratio for Whole Plant: 1 mL of extraction solvent per g of matrix Ratio for Grain: 2 mL of extraction solvent per g of matrix Ratio for Oilseed rape seed: 4 mL of extraction solvent per g of matrix Clean-up: Dispersive SPE with primary/secondary amine (PSA) Liquid/liquid partition: Addition of salt mixture Dilution with acetonitrile/water (1/1, v/v) Sample concentration in final extract: 0.05 g sample per mL of extract Method S22-06799 (Method 2):

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	Extraction: Methanol/water/hydrochloric acid (70/25/5 v/v). Ratio: 20 mL of extraction solvent per g of matrix Dilution with methanol/water (1/1 v/v) Sample concentration in final extract: 0.025 g sample per mL of extract Metabolism Method (Method 3): Extraction: Methanol Ratio: 20 mL of extraction solvent per g of matrix Dilution with water Sample concentration in final extract: 0.025 g sample per mL of extract															
Selectivity and Specificity	Demonstrated by evaluation of one (1) mass transitions for Method 1 and Method 2 and by validation of two (2) mass transitions for Method 3. Analyte levels or chromatographic interferences of unknown compounds in control sample extracts were in some cases above of what would correspond to an analyte level of 30% of the LOQ.															
Matrix Effects on Analyte Detection	For Method 1 Method 2: Matrix effects have been evaluated during the validation studies. For the Metabolism Method (evaluated during this study): Significant ($\geq 20\%$) for wheat whole plant. Insignificant ($< 20\%$) for wheat grain and oilseed rape seeds.															
Calibration	Matrix matched calibration standards A minimum of five (5) concentration levels Single determination Injection of standard solutions spread over the whole acquisition batch Method 1: 0.15 ng/mL to 10 ng/mL corresponding to 0.003 mg/kg to 0.20 mg/kg Method 2 and Method 3: 0.075 ng/mL to 7.5 ng/mL corresponding to 0.003 mg/kg to 0.30 mg/kg Coverage: 30% of the LOQ to at least + 20% of the highest analyte concentration level detected in a diluted sample extract. The validated range does not exceed two (2) orders of magnitude.															
Quantification	Linear regression with 1/x weighting Regression residuals randomly distributed. Correlation coefficients (R) ≥ 0.99															
Accuracy and Precision	The following fortification experiments were conducted: Method 1 and Method 2: One fortification at LOQ, one fortification at 10x LOQ and one fortification at 1000x LOQ Method 3: Five fortifications at LOQ, five fortifications at 10x LOQ and one fortification at 1000x LOQ Mean recoveries for the evaluated mass transitions comply with the standard acceptance criteria of the guidance document SANTE/2020/12830, rev. 2: <table><tr><th>Concentration Level (mg/kg)</th><th>Range of Mean Recoveries (%)</th><th>Precision, Rel. Std. Dev. (%)</th></tr><tr><td>≤ 0.01</td><td>60 - 120</td><td>≤ 30</td></tr><tr><td>$> 0.01 - \leq 0.1$</td><td>70 - 120</td><td>≤ 20</td></tr><tr><td>$> 0.1 - \leq 1.0$</td><td>70 - 110</td><td>≤ 15</td></tr><tr><td>> 1</td><td>70 - 110</td><td>≤ 10</td></tr></table>	Concentration Level (mg/kg)	Range of Mean Recoveries (%)	Precision, Rel. Std. Dev. (%)	≤ 0.01	60 - 120	≤ 30	$> 0.01 - \leq 0.1$	70 - 120	≤ 20	$> 0.1 - \leq 1.0$	70 - 110	≤ 15	> 1	70 - 110	≤ 10
Concentration Level (mg/kg)	Range of Mean Recoveries (%)	Precision, Rel. Std. Dev. (%)														
≤ 0.01	60 - 120	≤ 30														
$> 0.01 - \leq 0.1$	70 - 120	≤ 20														
$> 0.1 - \leq 1.0$	70 - 110	≤ 15														
> 1	70 - 110	≤ 10														
Stability of Analyte in Standard Solutions	Stability of analyte(s) in standard solutions has been demonstrated in Analytical Phase S22-01575-L1 [please refer to dRR part B7, KCP 7.2.3.3/01] for 177 days when prepared in methanol and stored at typically 1 °C to 10 °C in the dark.															
Extraction Efficiency	Two samples with incurred residues were analysed (three replicates) following the methods described with evaluation of one (1) mass transition. The results were compared to the results obtained using the extraction solvent systems from the metabolism study [Vol 3-B7, RAR 2018].															

Conclusion

The method based on the metabolism method (Method 3) was found to be valid according to the guidance document SANTE/2020/12830, rev. 2 for the determination of boscalid with an LOQ of 0.01 mg/kg for wheat grain and wheat whole plant and oilseed rape seeds. The extraction efficiency is considered sufficiently proven for the Multi-residue method (Method 1 [S22-00980]) and the data generation / risk assessment method (Method 2 [S22-06799]) compared to the metabolism method (Method 3) [Vol 3-B7, RAR 2018]. The following table shows the average extraction efficiency of each method (Method 1 and Method 2) and for each matrix when compared to the metabolism method (Method 3).

	Wheat (Whole Plant)	Wheat (Grain)	OSR (Seeds)
Method 1	89.8%	110%	88.0%
Method 2	91.1%	94.3%	101%

Therefore, the suitability of the extraction procedures from the Multi-residue method (Method 1 [S22-00980]) and the data generation / risk assessment method (Method 2 [S22-06799]) in wheat grain, wheat whole plant and oilseed rape seeds is demonstrated according to the criteria from the guideline SANTE/2017/10632. Rev.4.

A 2.1.1.2 Description of Methods for the Analysis of Water (KCP 5.1)

A 2.1.1.2.1 Analytical method 1

A 2.1.1.2.1.1 Method validation

Comments of zRMS:	Study is accepted
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Reference:	KCP 5.1.2/03
Report	Validation of Analytical Method for Determination of Active Ingredient Concentration and Stability of Boscalid in Matrix, following the Application of Boscalid 500 g/kg WG, Pandey P.K., 2023, JRF Study Number: 228-2-13-31234, Sponsor Study Number: UPL/2022/2648.
Guideline(s):	Yes, SANTE/2020/12830, Rev.1 (February 24, 2021)
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

This study was performed to validate the analytical method for determination of Boscalid 500 g/Kg WG concentration in matrix (reconstituted water, and 1X AAP), and to establish the stability of boscalid in matrix (reconstituted water, and 1X AAP) treated with Boscalid 500 g/Kg WG up to 48 h and 96 h by using HPLC.

The test item working solutions were prepared in reconstituted water and in 1X AAP. All the final working

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solutions prepared for the experiments matrix effect, specificity, linearity, limit of quantification (LOQ), recovery and repeatability (%RSD), stability (standard solution stability and test item stability), were injected onto HPLC instrument for analysis. The analytical method parameters are provided below:

Instrument: HPLC [Shimadzu, LC-2010CHT with Lab-Solution Software]
 Column: Agilent Eclipse, XDB-C-18 [150 mm x 4.6 mm (i.d.), 5.0 µm particle size]
 Mobile Phase: Acetonitrile (55%): Milli-Q Water (45%), v/v
 Injection Volume: 20 µL
 Flow Rate: 1.0 mL/minute
 Wavelength: 220 nm
 Column Temperature: 25°C
 Detector: UV
 Injector: Autosampler
 Retention Time: 5.5 minutes (approximately)

Results and discussions

The analytical method for the determination of Boscalid in matrix, following the application of Boscalid 500 g/kg WG was successfully validated with a limit of quantification (LOQ) of 4.39 mg/L in reconstituted water and 0.31 mg/L in 1X AAP medium. No additional confirmatory method or an ILV is required.

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

Matrix	Analyte	Fortification level (mg/kg) (n = 5)	Mean recovery (%)	RSD (%)	Comments
Reconstituted Water	Boscalid	LOQ Level	92.27	2.60	Range: 89.11-95.69
		High Dose Level	97.11	6.09	Range: 93.79-107.62
1X AAP	Boscalid	LOQ Level	107.94	1.07	Range: 106.48-109.23
		High Dose Level	82.56	0.8	Range: 81.89-83.42

Table A 6: Characteristics for the analytical method used for validation of boscalid residues in reconstituted water and 1X AAP

	Boscalid (in reconstituted water)	Boscalid (in 1X AAP)
Specificity	Blank value < 30 % LOQ)	Blank value < 30 % LOQ)
Calibration (type, number of data points)	Individual calibration data presented Calibration line equation presented 6 data points r = 0.993 Regression residuals presented in residual plot with random distribution	Individual calibration data presented Calibration line equation presented 6 data points r= 0.994 Regression residuals presented in residual plot with random distribution
Calibration range	0.09 – 9.11 mg/L	0.09 – 9.11 mg/L
Assessment of matrix effects is presented	Yes	Yes
Limit of determination/quantification	4.39 mg/L	0.31 mg/L

Conclusion

From the results of the validation, it is concluded that the method is sensitive, precise, and accurate for the analysis of the active ingredient concentration and stability of Boscalid 500 g/Kg WG in the media, viz., reconstituted water for the daphnia study, and 1X AAP media for the alga study. The test item was stable up to 96 h in 1X AAP media, and up to 48 h in reconstituted water (80% to 120% of the nominal concentration). The data meet the acceptance criteria of SANTE/2020/12830, Rev.2 for support of risk assessment data generation.

A 2.1.1.2.1.2 Confirmatory method (if required)

No confirmatory method is required.

A 2.1.1.2.2 Analytical method 2

A 2.1.1.2.2.1 Method validation

Comments of zRMS:	Study is accepted
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Reference:	KCP 5.1.2/04
Report	Boscalid 500 WG: Acute Contact and Oral Toxicity to Bumblebees (<i>Bombus terrestris</i> L.) in the Laboratory, Knautz. T., Kowalczyk, F., 2022a, Study No. 165091105, Sponsor Study No.: UPL/2022/2874.
Guideline(s):	Yes, SANTE/2020/12830, Rev.1 (February 24, 2021)
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

Duplicate samples from the sample list were taken from the biological ibacon study 165091105. The concentrations of the active ingredient boscalid were determined in one sample of each duplicate. The samples were stored deep frozen ($\leq -20^{\circ}\text{C}$) until analysis was performed.

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

Contact fortification solutions: The test item was dissolved in pure water with 0.1% Triton X-100 (sonicated for 5 minutes and stirred for 60 minutes) to get fortified samples of about 250 g test item/L and 125 g test item/L.

Oral fortification solutions (feeding solutions): The test item was dissolved in 50% w/v sucrose solution (sonicated for 5 minutes and stirred for 60 minutes) to get fortified samples of about 35 g test item/L and 17.5 g test item/L.

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Chromatographic Conditions: HPLC-Conditions:

System: Waters Acquity
 Software: Empower 3
 Column: Kinetex C18, 50 x 2.1 mm; 2.6 µm
 Temperature: 25°C
 Mobile Phase: 50% HPLC-water, 50% acetonitrile, isocratic
 Total Run Time: 2.5 minutes
 Flow Rate: 0.3 mL/min
 Injection Volume: 2 µL
 Detector: DA- Detector at 191 to 400 nm, monitoring wavelength at 205 nm
 Retention Time: Boscalid: 1.6 minutes

Results and discussions

The analytical method for the determination of boscalid in contact and oral test solutions was successfully validated with a limit of quantification (LOQ) of 125 g test item/L (corresponding to 6.4 mg boscalid/L after dilution by factor 100000) in contact and oral test stock solution and LOQ of 17.5 g test item/L (corresponding to 4.5 mg boscalid/L after dilution by factor 2000) in oral test feeding solution.

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

Matrix	Analyte	Fortification level (g/L) (n = 5)	Mean recovery (%)	RSD (%)	Comments
Contact Test	Boscalid	LOQ Level 125 test item/L	102	1	Range: 100-104
		Higher Level 300 test item/L	103	2	Range: 102-106
		Control	Boscalid not detectable		2 replicates
Oral Test Feeding Solution	Boscalid	LOQ Level 125 test item/L	101	1	Range: 100-103
		Higher Level 300 test item/L	103	1	Range: 101-103
		Control	Boscalid not detectable		2 replicates

Table A 8: Characteristics for the analytical method used for validation of boscalid residues in contact and oral test solution

	Boscalid (in Contact Test)	Boscalid (in Oral Test)
Specificity	Blank value < 30% LOQ)	Blank value < 30% LOQ)
Calibration (type, number of data points)	Individual calibration data presented Calibration line equation presented 7 data points r = 1.0000 Regression residuals presented in residual plot with random distribution and no visible trend	Individual calibration data presented Calibration line equation presented 7 data points r= 1.0000 Regression residuals presented in residual plot with random distribution and no visible trend

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	Boscalid (in Contact Test)	Boscalid (in Oral Test)
Specificity	Blank value < 30% LOQ)	Blank value < 30% LOQ)
Calibration range	1.5 to 20 mg Boscalid/L (corresponding to 23% of LOQ to 156% of higher fortification level)	1.0 to 15 mg Boscalid/L (corresponding to 22% of LOQ to 167% of higher fortification level)
Assessment of matrix effects is presented	Yes	Yes
Limit of determination/quantification	125 g test item/L (corresponding to 6.4 mg boscalid/L after dilution by factor 10000)	17.5 g test item/L (corresponding to 4.5 mg boscalid/L after dilution by factor 2000)

Conclusion

All validity criteria for the analytical method have been met. The data meet the acceptance criteria of SANTE/2020/12830, Rev.2 for support of risk assessment data generation.

A 2.1.1.2.2.2 Confirmatory method (if required)

No confirmatory method is required.

A 2.1.1.2.3 Analytical method 3

A 2.1.1.2.3.1 Method validation

Comments of zRMS:	Study is accepted
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Reference:	KCP 5.1.2/05
Report	Boscalid 500 WG: Chronic Oral Toxicity Test on the Honey Bee (<i>Apis mellifera</i> L.) in the Laboratory, Knautz, T., Kowalczyk, F., 2022b, Study No. 165091136, Sponsor Study Number: UPL/2022/2873.
Guideline(s):	Yes, SANTE/2020/12830, Rev.1 (February 24, 2021)
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

Samples were taken from the biological ibacon study 165091136. The concentrations of the active ingredient Boscalid were determined in one sample of Feeding Solution 19886 DAA6 and Feeding Solution 1023 DAA6. The samples were stored deep frozen ($\leq -20^{\circ}\text{C}$) until analysis was performed. Analysis was performed by HPLC-UV method.

Sample Preparation:

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After thawing, an aliquot of each sample was diluted with solvent mixture (Acetonitrile/pure water (50/50 v/v)) 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 (sonicated for 15 minutes and stirred for 30 minutes) to get fortified samples of about 60 g test item/L. These solutions were diluted in 50% w/v sucrose solution + 0.1% Xanthan to obtain fortified samples at approximately 1.0 g test item/L. Fortified samples were diluted with solvent mixture to match calibration range.

Replicates:

Five independent replicates per fortification level. Two independent replicates of matrix blanks.

HPLC-Conditions:

System: Waters Acquity
 Software: Empower 3
 Column: Kinetex C18, 50 x 2.1 mm; 2.6 µm
 Temperature: 25°C
 Mobile Phase: 50% HPLC-water / 50% acetonitrile, isocratic
 Total Run Time: 2.5 minutes
 Flow Rate: 0.3 mL/min
 Injection Volume: 2 µL
 Detector: DA- Detector at 191 to 398 nm, monitoring wavelength at 205 nm
 Retention Time: Boscalid: 1.6 minutes

Results and discussions

The analytical method for the determination of boscalid in Feeding Solution (19886 DAA6 and 1023 DAA6), was successfully validated with a limit of quantification (LOQ) of 1.0 g test item/L (corresponding to 5.1 mg boscalid/L after dilution by factor 100). No additional confirmatory method or an ILV is required.

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

Matrix	Analyte	Fortification level (g/L) (n = 5)	Mean recovery (%)	RSD (%)	Comments
Fortified sample (feeding solution)	Boscalid	LOQ Level (1.0 g test item/L)	103	8	Range: 92-112
		High Dose Level (1.0 g test item/L)	105	4	Range: 98-110
		Control	Boscalid not detectable		2 replicates
Feeding Solution 1023 DAA6	Boscalid	-	100 %	-	Recovery Rate of Nominal Value
Feeding Solution 19886 DAA6	Boscalid	-	108 %	-	Recovery Rate of Nominal Value

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Table A 10: Characteristics for the analytical method used for validation of boscalid residues in feeding solution

	Boscalid
Specificity	Blank value < 30% LOQ
Calibration (type, number of data points)	Individual calibration data presented Calibration line equation presented 6 data points $r = 0.9999$ Regression residuals presented in residual plot with random distribution and no visible trends
Calibration range	1.0 to 25 mg Boscalid/L (corresponding to 19% of LOQ to 162% of higher fortification level)
Assessment of matrix effects is presented	Yes No significant matrix effect ($\geq 20\%$) was observed
Limit of determination/quantification	LOD: 1.0 mg boscalid/L LOQ: 1.0 g test item/L corresponding to 5.1 mg boscalid/L after dilution by factor 100

Conclusion

All validity criteria for the analytical method have been met. The data meet the acceptance criteria of SANTE/2020/12830, Rev.2 for support of risk assessment data generation.

A 2.1.1.2.3.2 Confirmatory method (if required)

No confirmatory method is required.

A 2.1.1.2.4 Analytical method 4

A 2.1.1.2.4.1 Method validation

Comments of zRMS:	Study is accepted
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Reference: KCP 5.1.2/06
 Report Effects of BOSCALID 500 WG (FGG01) on honeybees (*Apis mellifera* L.) 22-day larval toxicity test with repeated exposure, Colli, M., 2022, Study No. BT115/22, Sponsor Study Number: UPL/2022/2796.
 Guideline(s): Yes, SANTE/2020/12830, Rev.1 (February 24, 2021)
 Deviations: No
 GLP: Yes

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Acceptability: Yes

Materials and methods

Reagents

Acetonitrile LC-MS Chromasolv $\geq 99.9\%$ Honeywell 34967
Water ultrapure filtered at 0.2 μm by Sartorius system (UP water)

Apparatus

Liquid Chromatograph: Agilent HPLC with DAD detector, 1200 series with Chemstation
Data analysis software: Open Lab CDS ChemStation Edition for LC & LC/MS System, version C.01.08 – Agilent Technologies [2001 – 2017]
Column: Poroshell 120 SB-C18 3 x 50 mm – 2.7 μm
Analytical balance: Mettler Toledo XP105DR
Pipettes: Gilson 10-5000 μL
Routine Laboratory glassware

Experimental conditions

Eluent A: Water
Eluent B: Acetonitrile
Eluent ratio A/B: 50/50
Flow: 0.4 mL/min
Injection volume: 1.0 μL
Column temp.: 30°C
Stop Time: 5.0 minutes
Analysed wavelength: 205 nm
Retention time: approx. 2.1 minutes

Results and discussions

The analytical method for the determination of Boscalid in aqueous matrix was successfully validated with a limit of quantification (LOQ) of 0.1 g boscalid/L.

No additional confirmatory method or an ILV is required.

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

Matrix	Analyte	Fortification level (g/L) ($n = 5$)	Mean recovery (%)	RSD (%)	Comments
Aqueous solution	Boscalid	LOQ Level (0.21 g boscalid /L)	99.47	1.31	Range: 97.2-100.53
		High Level (13.95 g boscalid/L)	104.25	1.85	Range: 100.97-105.8

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Table A 12: Characteristics for the analytical method used for validation of boscalid residues in aqueous solution

	Boscalid
Specificity	Blank value < 30% LOQ
Calibration (type, number of data points)	Individual calibration data presented Calibration line equation presented 5 data points $r = 0.9990$ Regression residuals presented in residual plot with random distribution and no visible trends
Calibration range	15.0144 mg/L – 500.4790 mg/L (corresponding to 0.0751 g/L – 16.6826 g/L in aqueous matrix)
Assessment of matrix effects is presented	Yes No significant matrix effect ($\geq 20\%$) was observed
Limit of determination/quantification	LOD: 15.0144 mg boscalid/L LOQ: 0.21 g boscalid/L

Conclusion

The analytical method for the determination of boscalid in aqueous matrix was validated according to SANTE/2020/12830 rev.1 guidance document. All the validity criteria were met. The data meet the acceptance criteria of SANTE/2020/12830, Rev.2 for support of risk assessment data generation.

A 2.1.1.2.4.2 Confirmatory method (if required)

No confirmatory method is required.

A 2.1.1.2.5 Analytical method 5

A 2.1.1.2.5.1 Method validation

Comments of zRMS:	Study is accepted
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Reference: KCP 5.1.2/07
 Report Boscalid 500 WG: Effects on Terrestrial (Non-Target) Plants: Seedling Emergence and Seedling Growth Test, Stürtz, S., Horstmann, C., 2022 a, Study No. 165091086, Sponsor Study Number: UPL/2022/0597.
 Guideline(s): Yes, SANTE/2020/12830, Rev.1 (February 24, 2021)
 Deviations: No
 GLP: Yes
 Acceptability: Yes

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Materials and methods

Samples were taken from the biological ibacon study 165091086. Analysis of Boscalid in the biological dosing solution was performed by HPLC-UV method.

Sample Preparation:

After thawing, an aliquot of each sample was diluted with solvent mixture (Acetonitrile/pure water (50/50 v/v)) to match the calibration range. Samples were diluted while some solutions were stirring. The samples were analysed on the day of processing.

Fortification Procedure:

The test item was dissolved in pure water (stirred for 45 minutes) to get fortified samples of about 25 g test item/L and 12.5 g test item/L. Fortified samples were diluted with solvent mixture to match calibration range.

Replicates:

Five independent replicates per fortification level. Two independent replicates of matrix blanks

HPLC-Conditions:

System: Waters Acquity
 Software: Empower 3
 Column: Kinetex C18, 50 x 2.1 mm; 2.6 µm
 Temperature: 25°C
 Mobile Phase: A: HPLC-water + 0.1% formic acid
 B: acetonitrile + 0.1% formic acid
 Total Run Time: 2 minutes
 Flow Rate: 0.3 mL/min
 Injection Volume: 2 µL
 Detector: DA- Detector at 191 to 400 nm, monitoring wavelength at 254 nm
 Retention Time: Boscalid: 0.99 minutes

Results and discussions

The analytical method for the determination of boscalid in biological dosing solution was successfully validated with a limit of quantification (LOQ) of 12.5 g test item/L (corresponding to 12.9 mg boscalid/L after dilution by factor 500). No additional confirmatory method or an ILV is required.

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

Matrix	Analyte	Fortification level (g/L) (n = 5)	Mean recovery (%)	RSD (%)	Comments
Fortified sample (biological dosing solution)	Boscalid	LOQ Level (12.5 g test item/L)	90	12	Range: 71-100
		High Dose Level (25 g test item/L)	96	2	Range: 94-99
		Control	Boscalid not detectable		2 replicates
Biological dosing solution (20 g test item/L)	Boscalid	-	99	-	Recovery Rate of Nominal Value

Table A 14: Characteristics for the analytical method used for validation of boscalid residues in biological dosing solution.

	Boscalid
Specificity	Blank value < 30 % LOQ
Calibration (type, number of data points)	Individual calibration data presented Calibration line equation presented 8 data points $r = 1.0000$ Regression residuals presented in residual plot with random distribution and no visible trends
Calibration range	2.5 to 35 mg boscalid/L (corresponding to 19% of LOQ to 136% of higher fortification level)
Assessment of matrix effects is presented	Yes No significant matrix effect ($\geq 20\%$) was observed
Limit of determination/quantification	LOD: 2.5 mg boscalid/L LOQ: 12.5 g test item/L corresponding to 12.9 mg boscalid/L after dilution by factor 500

Conclusion

All validity criteria for the analytical method have been met. The data meet the acceptance criteria of SANTE/2020/12830, Rev.2 for support of risk assessment data generation.

A 2.1.1.2.5.2 Confirmatory method (if required)

No confirmatory method is required.

A 2.1.1.2.6 Analytical method 6

A 2.1.1.2.6.1 Method validation

Comments of zRMS:	Study is accepted
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Reference:	KCP 5.1.2/08
Report	Boscalid 500 WG: Effects on Terrestrial (Non-Target) Plants: Vegetative Vigour Test, Stürtz, S., Horstmann, C., 2022 b, Study No. 165091087, Sponsor Study Number: UPL/2022/0598.
Guideline(s):	Yes, SANTE/2020/12830, Rev.1 (February 24, 2021)
Deviations:	No
GLP:	Yes
Acceptability:	Yes

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Materials and methods

Samples were taken from the biological ibacon study 165091087. Analysis of boscalid in the biological dosing solution was performed by HPLC-UV method.

Sample Preparation:

After thawing, an aliquot of each sample was diluted with solvent mixture (Acetonitrile/pure water (50/50 v/v)) 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 pure water (stirred for 45 minutes) to get fortified samples of about 25 g test item/L and 12.5 g test item/L. Fortified samples were diluted with solvent mixture to match calibration range.

Replicates:

Five independent replicates per fortification level.

Two independent replicates of matrix blanks

HPLC-Conditions:

System: Waters Acquity
 Software: Empower 3
 Column: Kinetex C18, 50 x 2.1 mm; 2.6 µm
 Temperature: 25°C
 Mobile Phase: A: HPLC-water + 0.1% formic acid
 B: acetonitrile + 0.1% formic acid

Total Run Time: 2 minutes
 Flow Rate: 0.3 mL/min
 Injection Volume: 2 µL
 Detector: DA- Detector at 191 to 400 nm, monitoring wavelength at 254 nm
 Retention Time: Boscalid: 0.99 minutes

Results and discussions

The analytical method for the determination of boscalid in the biological dosing solution was successfully validated with a limit of quantification (LOQ) of 12.5 g test item/L (corresponding to 12.9 mg boscalid/L after dilution by factor 500). No additional confirmatory method or an ILV is required.

Table A 15: Recovery results from method validation of boscalid using the analytical method

Matrix	Analyte	Fortification level (g/L) (n = 5)	Mean recovery (%)	RSD (%)	Comments
Fortified sample (biological dosing solution)	Boscalid	LOQ Level (12.5 g test item/L)	90	12	Range: 71-100
		High Dose Level (25 g test item/L)	96	2	Range: 94-99
		Control	Boscalid not detectable		2 replicates

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Matrix	Analyte	Fortification level (g/L) (n = 5)	Mean recovery (%)	RSD (%)	Comments
Biological dosing solution (20 g test item/L)	Boscalid	-	99	-	Recovery Rate of Nominal Value

Table A 16: Characteristics for the analytical method used for validation of Boscalid residues in biological dosing solution

	Boscalid
Specificity	Blank value < 30% LOQ
Calibration (type, number of data points)	Individual calibration data presented Calibration line equation presented 8 data points r = 1.0000 Regression residuals presented in residual plot with random distribution and no visible trends
Calibration range	2.5 to 35 mg boscalid/L (corresponding to 19% of LOQ to 136% of higher fortification level)
Assessment of matrix effects is presented	Yes No significant matrix effect ($\geq 20\%$) was observed
Limit of determination/quantification	LOD: 2.5 mg boscalid/L LOQ: 12.5 g test item/L corresponding to 12.9 mg boscalid/L after dilution by factor 500

Conclusion

All validity criteria for the analytical method have been met. The data meet the acceptance criteria of SANTE/2020/12830, Rev.2 for support of risk assessment data generation.

A 2.1.1.2.6.2 Confirmatory method (if required)

No confirmatory method is required.

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

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

No new or additional studies have been submitted.

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

No new or additional studies have been submitted.

A 2.1.2.2.1 Analytical method 1

A 2.1.2.2.1.1 Method validation

Comments of zRMS:	Study is accepted
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Reference:	KCP 5.2/01
Report	Validation of the Analytical Method for the Determination of Residues of Boscalid in Honey, Report Amendment 1, Sahvorost, N., 2022, Study No. S22-00776, Sponsor Study No. UPL/2022/0354.
Guideline(s):	Yes, SANTE/2020/12830, Rev.1 (February 24, 2021)
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

Samples of honey were extracted with acetonitrile, after addition of water. Clean-up was carried out by partition into acetonitrile followed by dispersive SPE with PSA. Quantification was performed by use of LC-MS/MS detection.

Results and discussions

The method was successfully validated for the determination of boscalid in honey from the tested LOQ of 0.01 mg/kg up to 0.1 mg/kg according to the guidance document SANTE/2020/12830, rev. 1 for risk assessment and/or monitoring.

Table A 17: Recovery results from method validation of boscalid using the analytical method

Matrix	Analyte	Fortification level (mg/kg) (n = 5)	Mean recovery (%)	RSD (%)	Comments
Mass Transition m/z 343 to 307 (Proposed for Quantification)					
Honey	Boscalid	0.01	98	2	Range: 96-100
		0.1	101	1	Range: 100-102

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Matrix	Analyte	Fortification level (mg/kg) (n = 5)	Mean recovery (%)	RSD (%)	Comments
Mass Transition m/z 343 to 307 (Proposed for Quantification)					
Mass Transition m/z 343 to 271 (Proposed for Confirmation)					
Honey	Boscalid	0.01	101	3	Range: 96-105
		0.1	102	1	Range: 101-104

Table A 18: Characteristics for the analytical method used for validation of boscalid residues in honey

	Boscalid
Specificity	Mass spectrum is provided (2 mass transitions were evaluated) Blank value < 30% LOQ)
Calibration (type, number of data points)	Individual calibration data presented Calibration line equation presented 8 data points $r \geq 0.995$ Regression residuals presented in residual plot with random distribution and no visible trends
Calibration range	0.06 ng/mL to 6 ng/mL (corresponds to 0.003 mg/kg to 3 mg/kg) Covering 30% LIQ to 20% highest calibration level
Assessment of matrix effects is presented	Yes No significant matrix effect ($\geq 20\%$) was observed
Limit of determination/quantification	LOD: 0.003 mg/kg LOQ: 0.01 mg/kg in honey for the two (2) mass transitions
Stability of Analyte in Standard Solutions	Boscalid was found to be stable for at least 12 days when prepared in acetonitrile and stored at typically 1°C to 10°C in the dark.
Stability of Analyte in Sample Extracts	Boscalid was found to be stable in final extracts of honey for 9 days when stored at typically 1°C to 10°C in the dark.

Conclusion

The method was successfully validated for the determination of boscalid in honey from the tested LOQ of 0.01 mg/kg up to 0.1 mg/kg according to the guidance document SANTE/2020/12830, rev. 1 for risk assessment and/or monitoring. The data meet the acceptance criteria of SANTE/2020/12830, Rev.2 for support of risk assessment data generation and monitoring purposes.

A 2.1.2.2.1.2 Independent laboratory validation

Comments of zRMS:	Study is accepted
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Reference: KCP 5.2/02
Report: Independent Laboratory Validation of the Analytical method for Determination of Residues of Boscalid in Honey, Rastogi, T., 2022, Study No. S22-00783, Sponsor Study No. UPL/2022/0845.
Guideline(s): Yes, SANTE/2020/12830, Rev.1 (February 24, 2021)
Deviations: No
GLP: Yes
Acceptability: Yes

Materials and methods

The principle of the analytical procedure is in line with the primary method:
Homogenisation: Thoroughly stirring with spatula
Extraction: Acetonitrile and addition of water
Liquid/Liquid Partition: Adding content of Citrate-Kit-1 (Bekolut, CK-01-050)
Clean-up: Transferring an aliquot to PSA-Kit-01 (Bekolut, PK-01)
Dilution: DF 10 in methanol/water (1/1, v/v) containing 0.5% formic acid
Quantification: LC-MS/MS

Results and discussions

The method for the determination of boscalid in honey was independently validated according to the guidance document SANTE/2020/12830, rev. 1.

Table A 19: Recovery results from independent laboratory validation of boscalid using the analytical method

Matrix	Analyte	Fortification level (mg/kg) (n = 5)	Mean recovery (%)	RSD (%)	Comments
Mass Transition m/z 343 to 307 (Proposed for Quantification)					
Honey	Boscalid	0.01	102	3.3	Range: 99.3-108
		0.1	99.8	1.3	Range: 98.0-101
Mass Transition m/z 343 to 271 (Proposed for Confirmation)					
Honey	Boscalid	0.01	99.6	2.6	Range: 98.5-104
		0.1	98.2	1.6	Range: 96.5-100

Table A 20: Characteristics for the analytical method used for independent laboratory validation of boscalid residues in honey

	Boscalid
Specificity	Mass spectrum is provided (2 mass transitions were

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	Boscalid
	evaluated) Blank value < 30% LOQ)
Calibration (type, number of data points)	Individual calibration data presented Calibration line equation presented 8 data points $r \geq 0.99$ Regression residuals presented in residual plot with random distribution and no visible trends
Calibration range	0.06 ng/mL to 6 ng/mL (corresponds to 0.003 mg/kg to 3 mg/kg) Covering 30% LOQ to 20% highest calibration level
Assessment of matrix effects is presented	Yes No significant matrix effect ($\geq 20\%$) was observed
Limit of determination/quantification	LOD: 0.003 mg/kg LOQ: 0.01 mg/kg in honey for the two (2) mass transitions
Stability of Analyte in Standard Solutions	The stability of the analyte in working solutions, e.g. stock, fortification and calibration solutions prepared in respective solvent is demonstrated in the method validation study.
Stability of Analyte in Sample Extracts	The stability of the analyte in the final extracts are demonstrated in the method validation study.

Conclusion

The method was successfully independently validated for the determination of boscalid in/on honey from the tested LOQ of 0.01 mg/kg according to the guidance document(s) SANTE/2020/12830, rev. 1 for risk assessment and/or monitoring.

No addition or modification to the original method other than optimisation of instrumental parameters was done. Primary validation and independent laboratory validation were carried out at different locations and by different study personnel. No communication with the method developers or others familiar with the method was necessary to carry out the analysis. The analytical method meets the requirements of guidance document(s) SANTE/2020/12830, rev. 2.

A 2.1.2.2.1.3 Confirmatory method

No confirmatory method is required (two mass transitions were monitored).

A 2.1.2.2.1.4 Extraction efficiency

Not applicable.

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

No new or additional studies have been submitted.

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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

A 2.1.2.4.1.1 Independent laboratory validation

Comments of zRMS:	Study is not accepted as ILV
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Reference: KCP 5.2/03

Report Independent Laboratory Validation of an Analytical Method for Determination of Boscalid and its Metabolites M510F47, M510F49 and M510F64 in Ground and Drinking Water, Kaiser, M., 2023, Study No. S22-01028, Sponsor Study No. UPL/2022/21136.

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

Deviations: No

GLP: Yes

Acceptability: Yes

Materials and methods

The principle of the analytical procedure is in line with the primary method:
 Homogenisation: Shaker
 Quantification: LC-MS/MS

Results and discussions

The method for the determination of boscalid in drinking water was independently validated according to the guidance document SANTE/2020/12830, rev. 1.

Table A 21: Recovery results from independent laboratory validation of boscalid using the analytical method

Matrix	Analyte	Fortification level (µg/L) (n = 5)	Mean recovery (%)	RSD (%)	Comments
Mass Transition m/z 343 to 307 (Proposed for Quantification)					
Ground water	Boscalid	0.1	119	4	Range: 114-126
		1.0	119	2	Range: 116-122
Drinking water	Boscalid	0.1	106	3	Range: 103-111
		1.0	102	3	Range: 98-106
Mass Transition m/z 343 to 271 (Proposed for Confirmation)					
Ground water	Boscalid	0.1	117	4	Range: 112-121

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Matrix	Analyte	Fortification level (µg/L) (n = 5)	Mean recovery (%)	RSD (%)	Comments
		1.0	119	2	Range: 117-122
Drinking water	Boscalid	0.1	105	2	Range: 103-109
		1.0	104	3	Range: 100-107

Table A 22: Characteristics for the analytical method used for independent laboratory validation of boscalid residues in water

	Boscalid
Specificity	Mass spectrum is provided (2 mass transitions were evaluated) Blank value < 30 % LOQ)
Calibration (type, number of data points)	Individual calibration data presented Calibration line equation presented 9 data points r = 0.9995 Regression residuals presented in residual plot with random distribution and no visible trends
Calibration range	Concentration range: 0.03 ng/mL to 2.0 ng/mL Corresponding mass fraction range: 0.03 µg/L to 2.0 µg/L Coverage: 30% of the LOQ to at least + 20% of the highest analyte concentration level detected in a sample extract
Assessment of matrix effects is presented	Yes No significant matrix effect (≥ 20%) was observed
Limit of determination/quantification	LOD: 0.03 µg/L LOQ: 0.1 µg/L

Conclusion

The method was successfully independently validated for the determination of boscalid and its metabolites M510F49, M510F64 and M510F47 in ground and drinking water from the tested LOQ of 0.1 µg/L up to 1.0 µg/L according to the guidance document SANTE/2020/12830, rev. 1 for monitoring. No addition or modification to the original method ther than optimisation of instrumental parameters was done. Primary validation and independent laboratory validation were carried out at different locations and by different study personnel No communication with the method developers or others familiar with the method was necessary to carry out the analysis and the first attempt made resulted in the validation data reported here.

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

No new or additional studies have been submitted

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

A 2.1.2.6.1 Analytical method 1

A 2.1.2.6.1.1 Method validation

Comments of zRMS:	Study is accepted
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Reference:	KCP 5.2/04
Report	Development and Validation of an Multi-Residue Method QuEChERS for the Determination of Boscalid and its Metabolite M510F01 in Body Fluids, Rastogi T., 2022, Study No. S22-00982, Sponsor Study No. UPL/2022/2054.
Guideline(s):	Yes, SANTE/2020/12830, Rev.1 (February 24, 2021)
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

The objective of the study was to validate an analytical method for the determination of boscalid and its metabolite M510F01 in/on body fluids (blood and urine) in accordance to guidance document SANTE/2020/12830, rev.1 for monitoring.

The limit of quantification was 0.01 mg/L for both analytes in both investigated matrices.

Samples of investigated matrices, after addition of water to bovine blood and human urine, were extracted with acetonitrile. The samples were shaken using a horizontal shaker. A SupelQuE Citrate Tube (55227-U) containing mixture of magnesium sulphate, sodium chloride, sodium citrate dibasic sesquihydrate and sodium citrate tribasic dehydrate was added. The extract was shaken and centrifuged. An aliquot of organic layer was transferred to another tube containing primary secondary amine (PSA, 55228-U). The samples were shaken and centrifuged. The samples were diluted in acetonitrile/water (1/1, v/v) containing 0.1% formic acid prior to the LC-MS/MS analysis for the determination of boscalid and M510F01.

Results and discussions

The method was successfully validated for the determination of boscalid and M510F01 in body fluids with LOQ of 0.01 mg/L according to the guidance document SANTE/2020/12830, rev. 1 for risk assessment and/or monitoring.

Table A 23: Recovery results from method validation of boscalid and metabolite M510F01 using the analytical method

Matrix	Analyte	Fortification level (mg/kg) (n = 5)	Mean recovery (%)	RSD (%)	Comments
Mass Transition m/z 343 to 307 (Proposed for Quantification)					

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Matrix	Analyte	Fortification level (mg/kg) (n = 5)	Mean recovery (%)	RSD (%)	Comments
Bovine Blood	Boscalid	0.01	112	2.0	Range: 109-115
		0.1	112	4.5	Range: 108-121
Human Urine	Boscalid	0.01	114	2.0	Range: 112-118
		0.1	116	2.0	Range: 115-120
Mass Transition m/z 343 to 140 (Proposed for Confirmation)					
Bovine Blood	Boscalid	0.01	116	4.2	Range: 109-120
		0.1	115	5.1	Range: 109-125
Human Urine	Boscalid	0.01	115	2.7	Range: 112-120
		0.1	115	2.8	Range: 111-119
Mass Transition m/z 359 to 323 (Proposed for Quantification)					
Bovine Blood	M510F01	0.01	107	3.9	Range: 102-112
		0.1	107	4.4	Range: 102-114
Human Urine	M510F01	0.01	109	5.8	Range: 104-119
		0.1	109	2.4	Range: 105-112
Mass Transition m/z 359 to 140 (Proposed for Confirmation)					
Bovine Blood	M510F01	0.01	109	2.8	Range: 105-113
		0.1	108	4.4	Range: 102- 116
Human Urine	M510F01	0.01	109	2.7	Range: 106 - 112
		0.1	109	2.9	Range: 106-113

Table A 24: Characteristics for the analytical method used for validation of boscalid and M510F01 residues in body fluids

	Boscalid	M510F01
Specificity	Mass spectrum is provided (2 mass transitions were evaluated) Blank value < 30% LOQ)	Mass spectrum is provided (2 mass transitions were evaluated) Blank value < 30% LOQ)
Calibration (type, number of data points)	Individual calibration data presented Calibration line equation presented 6 data points $r \geq 0.99$ Regression residuals presented in residual plot with random distribution and no visible trends	Individual calibration data presented Calibration line equation presented 6 data points $r \geq 0.99$ Regression residuals presented in residual plot with random distribution and no visible trends
Calibration range	Concentration range: 0.15 ng/mL to 10 ng/mL Corresponding mass fraction range: 0.003 mg/L to 0.20 mg/L Coverage: 30% of the LOQ to at least + 20% of the highest analyte	Concentration range: 0.15 ng/mL to 10 ng/mL Corresponding mass fraction range: 0.003 mg/L to 0.20 mg/L Coverage: 30% of the LOQ to at least + 20% of the highest analyte

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	Boscalid	M510F01
	concentration level detected in a sample extract	concentration level detected in a sample extract
Assessment of matrix effects is presented	Yes No significant matrix effect ($\geq 20\%$) was observed	Yes No significant matrix effect ($\geq 20\%$) was observed
Limit of determination/quantification	LOD: 30% of the LOQ (lowest calibration standard) LOQ: 0.01 mg/L	LOD: 30% of the LOQ (lowest calibration standard) LOQ: 0.01 mg/L
Stability of Analyte in Standard Solutions	The stability of the working solution (i.e. stock, fortification and intermediate calibration solutions) of boscalid in methanol was demonstrated for 30 days when stored at 1°C to 10°C in the dark in the study S22-00979.	The stability of the working solution (i.e. stock, fortification and intermediate calibration solutions) of M510F01 in methanol was demonstrated for 9 days when stored at 1°C to 10°C in the dark.
Stability of Analyte in Sample Extracts	Recoveries within 70% - 120% in all matrix extracts for 8 days when stored at typically 1°C to 10°C in the dark	Recoveries within 70% - 120% in all matrix extracts for 8 days when stored at typically 1°C to 10°C in the dark

Conclusion

The method was successfully validated for the determination of boscalid and M510F01 in body fluids with LOQ of 0.01 mg/L up to 0.1 mg/kg according to the guidance document SANTE/2020/12830, rev. 1 for monitoring. The data meet the acceptance criteria of SANTE/2020/12830, Rev.2 for support of risk assessment data generation and monitoring purposes.

A 2.1.2.6.1.2 Confirmatory method

No confirmatory method is required (two mass transitions were monitored)

A 2.1.2.6.1.3 Extraction efficiency

Not applicable

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

No new or additional studies have been submitted