

REGISTRATION REPORT

Part B

Section 5

Analytical Methods

Detailed summary of the risk assessment

Product name(s): **INTUITY PLUS**

(Mandestrobin 40 SC)

Chemical active substance:
Mandestrobin, 400 g/L

Central Zone

Zonal Rapporteur Member State: Poland

CORE ASSESSMENT

(authorization)

Applicant: XXXX

Submission date: February 2024

Evaluation date: January 2025

Finalisation date: August 2025

Version history

When	What
February 2024	Article 33 submission – Initial Applicant's version
May 2024	<ul style="list-style-type: none"> - Update of the cover page with the product trade name 'Intuity Plus'. Mandestrobin 40 SC is the internal unique name. The internal name Mandestrobin 40 SC is the one used across the dRR content. - Update of Appendix 1: studies source and owner updated
January 2025	Initial RR by zRMS
April 2025	<p>The applicant's updates:</p> <ul style="list-style-type: none"> -Update of Table 5.1-1 with reference to method validation KCP 5.1.2/22 -Update of Appendix 1: reference for KCP 5.1.2/22 -Update of Appendix 2: method validation summary KCP 5.1.2/22 -Update of residue methods: 5.3.2.5 Description of methods for the analysis of water (KCP 5.2) and 5.3.2.7 Description of methods for the analysis of body fluids and tissues (KCP 5.2)
August 2025	Finalization of RR (fRR)

Table of Contents

5	Analytical methods.....	4
5.1	Conclusion and summary of assessment.....	4
5.2	Methods used for the generation of pre-authorization data (KCP 5.1).....	4
5.2.1	Analysis of the plant protection product (KCP 5.1.1)	4
5.2.1.1	Determination of active substance and/or variant in the plant protection product (KCP 5.1.1).....	4
5.2.1.2	Description of analytical methods for the determination of relevant impurities (KCP 5.1.1).....	6
5.2.1.3	Description of analytical methods for the determination of formulants (KCP 5.1.1)	8
5.2.1.4	Applicability of existing CIPAC methods (KCP 5.1.1).....	8
5.2.2	Methods for the determination of residues (KCP 5.1.2).....	8
5.3	Methods for post-authorization control and monitoring purposes (KCP 5.2)	12
5.3.1	Analysis of the plant protection product (KCP 5.2)	12
5.3.2	Description of analytical methods for the determination of residues of mandestrobin (KCP 5.2)	12
5.3.2.1	Overview of residue definitions and levels for which compliance is required	12
5.3.2.2	Description of analytical methods for the determination of residues in plant matrices (KCP 5.2).....	13
5.3.2.3	Description of analytical methods for the determination of residues in animal matrices (KCP 5.2).....	14
5.3.2.4	Description of methods for the analysis of soil (KCP 5.2).....	14
5.3.2.5	Description of methods for the analysis of water (KCP 5.2).....	15
5.3.2.6	Description of methods for the analysis of air (KCP 5.2).....	15
5.3.2.7	Description of methods for the analysis of body fluids and tissues (KCP 5.2)	15
5.3.2.8	Other studies/ information	16
Appendix 1	Lists of data considered in support of the evaluation.....	17
Appendix 2	Detailed evaluation of submitted analytical methods	26
A 2.1	Analytical methods for mandestrobin	26
A 2.1.1	Methods used for the generation of pre-authorization data (KCP 5.1).....	26
A 2.1.2	Methods for post-authorization control and monitoring purposes (KCP 5.2)	64

5 Analytical methods

5.1 Conclusion and summary of assessment

Sufficiently sensitive and selective analytical methods are **not** available for the active substance(s) and relevant impurities in the plant protection product.

Noticed data gaps are: **none.**

- ~~data gap 1~~
- ~~data gap 2~~
- ~~data gap 3~~

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

In the context of the residue methods the applicant's dRR was not rewritten. In the resulting zRMS' RR all comments /corrections/ add-ons were placed on the grey background.

Analytical methods are available in the DAR and this dossier. They are validated for the determination of residues of mandestrobin in plants (acceptable primary, confirmatory and ILVs in high water, high acid, high oil, high protein/starch (dry) crops), soil (acceptable primary and confirmatory method), water (acceptable primary and confirmatory method in surface water and in drinking water) and air (acceptable primary and confirmatory method). ~~Analytical methods for the determination of residues of mandestrobin in animal matrices are not necessary as no residues are to be expected in these matrices and no MRLs are proposed (MRLs set at the LOQ level). There is no residue definition for mandestrobin in body fluids and tissues therefore no methods are required.~~

Noticed data gaps are:

- ~~1. ILV of the analytical method of mandestrobin in drinking water.~~
- ~~2. Analytical methods for the determination of residues of mandestrobin in body fluids and tissues.~~

None (the gaps were closed).

Commodity/crop	Supported/ Not supported
OSR	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 mandestrobin in plant protection product Mandestrobin 40SC is provided as follows:

Comments of zRMS:	Accepted
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Report	Validation of analytical HPLC methods for the determination of active substance content and isomer ratio in a suspension concentrate (SC) containing mandestrobin, Lecocq, V., 2023, Report No.: 25587, Document No.: ROA-0088
Guideline(s):	SANCO/3030/99 rev.5
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

Mandestrobin (sum of isomers) - Samples of Mandestrobin 40SC (250 mg) are weighed into conical flasks, internal standard solution (10 mL, 12 g/L n-butyl benzoate in acetonitrile) is added at 20±1°C, acetonitrile (90 mL) is added and the flask is ultrasonicated for 10 minutes. The solution is mixed thoroughly and filtered (0.2 µm filter). Samples are analysed by HPLC-UV. The chromatographic conditions used are an Agilent Zorbax Eclipse XDB-C18 column (150 mm x 4.6 mm, 5 µm), isocratic elution with a mobile phase of acetonitrile/water/phosphoric acid (500/500/1, v/v/v) and detection at 275 nm. Quantification is achieved by use of an internal standard.

Mandestrobin S-isomer and mandestrobin R-isomer - Samples of Mandestrobin 40SC (65 mg) are weighed into conical flasks, xylene (25 mL) is added and the flask is ultrasonicated for 10 minutes. The solution is mixed thoroughly and filtered (0.45 µm filter). Samples are analysed by HPLC-UV. The chromatographic conditions used are a Phenomenex Sumichiral OA-4900 column (250 mm x 4.6 mm, 5 µm), isocratic elution with a mobile phase of n-hexane/ethanol (97/3, v/v) and detection at 275 nm. Quantification is achieved by use of an internal standard.

Validation - Results and discussions

Table 5.2-1: Methods suitable for the determination of active substance mandestrobin (sum of isomers), mandestrobin S-isomer and mandestrobin R-isomer in plant protection product Mandestrobin 40SC

	Mandestrobin (sum of isomers)	Mandestrobin S-isomer	Mandestrobin R-isomer
Author(s), year	Lecocq, V., 2023	Lecocq, V., 2023	Lecocq, V., 2023
Principle of method	HPLC-UV	HPLC-UV	HPLC-UV
Linearity (linear between mg/L / % range of the declared content) (correlation coefficient, expressed as r)	473 – 1409 µg/mL (equivalent to 18.9 - 56.4% w/w or 189 - 564 g/kg or 205 - 611 g/L mandestrobin corresponding to 51 - 151% of the concentration in the test item) $r^2 = 0.9999$ slope = 0.0011 intercept = 0.0065 5 levels	602 – 1399 µg/mL (equivalent to 23.2 - 53.8% w/w or 232 - 538 g/kg or 251 - 582 g/L mandestrobin corresponding to 62 - 144% of the concentration in the test item) $r^2 = 0.9991$ slope = 1.8539 intercept = 19.2441 5 levels	602 – 1399 µg/mL (equivalent to 23.2 - 53.8% w/w or 232 - 538 g/kg or 251 - 582 g/L mandestrobin corresponding to 62 - 144% of the concentration in the test item) $r^2 = 0.9996$ slope = 1.8292 intercept = 37.7243 5 levels
Precision – Repeatability Mean n = 6 (%RSD)	Sample set 1 %RSD: 0.30% Horrat, H _r : 0.19	Sample set 1 %RSD: 0.10 Horrat, H _r : 0.07	Sample set 1 %RSD: 0.10 Horrat, H _r : 0.07

	Mandestrobin (sum of isomers)	Mandestrobin S-isomer	Mandestrobin R-isomer
	Mean content: 371.2 g/kg Sample set 2 %RSD: 1.06% Horrat, H _r : 0.68 Mean content: 376.7 g/kg	Mean % area: 49.99% area Sample set 2 %RSD: 0.40 Horrat, H _r : 0.27 Mean content: 50.01% area	Mean % area: 50.01% area Sample set 2 %RSD: 0.40 Horrat, H _r : 0.27 Mean content: 50.00% area
Accuracy n = 2 (% Recovery)	292.9 g/kg (80% of target concentration) Mean recovery: 100.7% 369.3 g/kg (100% of target concentration) Mean recovery: 99.9% 440.4 g/kg (120% of target concentration) Mean recovery: 101.6% Overall recovery: 100.7% Overall RSD: 0.79%	295.7 g/kg (80% of target concentration) Mean recovery: 99.7% 369.0 g/kg (100% of target concentration) Mean recovery: 100.1% 443.6 g/kg (120% of target concentration) Mean recovery: 99.9% Overall recovery: 99.9% Overall RSD: 0.20%	295.7 g/kg (80% of target concentration) Mean recovery: 100.3% 369.0 g/kg (100% of target concentration) Mean recovery: 99.9% 443.6 g/kg (120% of target concentration) Mean recovery: 100.2% Overall recovery: 100.1% Overall RSD: 0.20%
Interference/ Specificity	There was no interference (>3%) from the blank formulation, reagent blank or internal standard solution. Peak identity confirmed by retention time and UV spectrum match with reference standard material.	There was no interference (>3%) from the blank formulation, the reagent blank or between the individual isomers. Peak identity confirmed by retention time and UV spectrum match with reference standard material.	There was no interference (>3%) from the blank formulation, the reagent blank or between the individual isomers. Peak identity confirmed by retention time and UV spectrum match with reference standard material.
Comment	Fully validated in accordance with SANCO/3030/99 rev. 5	Fully validated in accordance with SANCO/3030/99 rev. 5	Fully validated in accordance with SANCO/3030/99 rev. 5

Conclusion

The method is fully validated, for the determination of mandestrobin (sum of isomers), mandestrobin S-isomer and mandestrobin R-isomer in the plant protection product Mandestrobin 40SC, in accordance with SANCO/3030/99 rev. 5.

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

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

Comments of zRMS:	Accepted CIPAC method MT 198 was used for. Therefore no validation is required anyway.
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Reference:	KCP 5.1.1/02
Report	Validation of analytical GC-MS method for the determination of xylene isomers and ethylbenzene content in a suspension concentrate (SC) containing mandestrobin, Lecocq, V., 2023, Report No.: 25588, Document No.: ROA-0089
Guideline(s):	SANCO/3030/99 rev.5
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

Samples of Mandestrobin 40SC (~50.0 mg) are accurately weighed into headspace vials, DMSO (2 mL) and internal standard solution (2 mL, 50 µg/mL toluene in DMSO) are added. Samples are analysed by GC-MS. The chromatographic conditions used are a TR-WAX (100% polyethylene glycol) capillary column (30 m x 0.25 mm, i.d. 1.0 µm film thickness) with helium as the carrier gas and MS in EI mode (for xylenes fragment ions m/z 91, m/z 105, m/z 106, for ethyl benzene fragment ions m/z 65, m/z 91, m/z 106). Quantification is achieved by use of an internal standard (fragment ions m/z 65, m/z 91, m/z 92).

Validation - Results and discussions

Table 5.2-2: Methods suitable for the determination of the relevant impurities in plant protection product (PPP) Mandestrobin 40SC

	xylenes (ortho, meta, para), ethyl benzene max. 2.4 g/L in PPP			
	ortho-xylene	meta-xylene	para-xylene	ethyl benzene
Author(s), year	Lecocq, V., 2023			
Principle of method	GC-MS			
Linearity (linear between mg/L) (correlation coefficient, expressed as r)	0 – 50 µg/mL (equivalent to ca. 0 – 4 g/kg in formulation) $r^2 = 0.9997$ slope = 4.1324 intercept = -0.0068 6 levels	0 – 50 µg/mL (equivalent to ca. 0 – 4 g/kg in formulation) $r^2 = 0.9999$ slope = 5.694 intercept = -0.0049 6 levels	0 – 50 µg/mL (equivalent to ca. 0 – 4 g/kg in formulation) $r^2 = 0.9999$ slope = 5.6367 intercept = -0.005 6 levels	0 – 50 µg/mL (equivalent to ca. 0 – 4 g/kg in formulation) $r^2 = 1.0000$ slope = 5.7902 intercept = -0.0036 6 levels
Precision – Repeatability Mean n = 6 (%RSD)	%RSD: 3.76 Horrat, H_r : 0.74 Mean content: 0.137 g/kg ortho-xylene in Mandestrobin 40SC	%RSD: 3.09 Horrat, H_r : 0.60 Mean content: 0.134 g/kg meta-xylene in Mandestrobin 40SC	%RSD: 3.32 Horrat, H_r : 0.64 Mean content: 0.118 g/kg para-xylene in Mandestrobin 40SC	%RSD: 3.30 Horrat, H_r : 0.65 Mean content: 0.140 g/kg ethyl benzene in Mandestrobin 40SC
	%RSD: 3.25 Horrat, H_r : 0.78 Mean content: 0.528 g/kg sum of xylenes (ortho, meta, para), ethyl benzene in Mandestrobin 40SC			
Accuracy n = 6	0.101 g/kg ortho-xylene in Mandestrobin 40SC	0.099 g/kg meta-xylene in Mandestrobin 40SC Mean recovery: 97.1%	0.094 g/kg para-xylene in Mandestrobin 40SC Mean recovery: 97.1%	0.102 g/kg ethyl benzene in Mandestrobin 40SC

	xylenes (ortho, meta, para), ethyl benzene max. 2.4 g/L in PPP			
	ortho-xylene	meta-xylene	para-xylene	ethyl benzene
(% Recovery)	Mean recovery: 96.6% RSD: 3.30% 1.310 g/kg ortho-xylene in Mandestrobin 40SC Mean recovery: 95.8% RSD: 1.24%	RSD: 3.98% 1.287 g/kg meta-xylene in Mandestrobin 40SC Mean recovery: 100.2% RSD: 1.13%	RSD: 4.94% 1.223 g/kg para-xylene in Mandestrobin 40SC Mean recovery: 98.6% RSD: 2.05%	Mean recovery: 97.2% RSD: 4.27% 1.331 g/kg ethyl benzene in Mandestrobin 40SC Mean recovery: 100.3% RSD: 1.29%
	0.396 g/kg sum of xylenes (ortho, meta, para), ethyl benzene in Mandestrobin 40SC Mean recovery: 97.0% RSD: 2.97% 5.152 g/kg sum of xylenes (ortho, meta, para), ethyl benzene in Mandestrobin 40SC Mean recovery: 98.7% RSD: 1.37%			
Interference/ Specificity	There was no interference (>3%) from the blank formulation, reagent blank or internal standard blank solution. Peak identity confirmed by retention time and MS spectrum match with reference standard material and MS available from literature.			
LOQ	0.101 g/kg	0.099 g/kg	0.094 g/kg	0.102 g/kg
Comment	Fully validated in accordance with SANCO/3030/99 rev. 5	Fully validated in accordance with SANCO/3030/99 rev. 5	Fully validated in accordance with SANCO/3030/99 rev. 5	Fully validated in accordance with SANCO/3030/99 rev. 5

Conclusion

The method is fully validated, for the determination of xylenes (ortho, meta, para) and ethyl benzene in the plant protection product Mandestrobin 40SC, in accordance with SANCO/3030/99 rev. 5.

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

Not required, as the plant protection product contains no relevant co-formulants.

5.2.1.4 Applicability of existing CIPAC methods (KCP 5.1.1)

Not applicable.

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

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

Component of residue definition: mandestrobin				
Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing / EU agreed
Fresh water (Ecotoxicology)	Primary	0.0500 mg/L	HPLC-UV	Urann, K., 2016, Report No ROW-0096, Appendix 2, KCP 5.1.2/02
Seawater (Ecotoxicology)	Primary	0.00500 mg/L	HPLC-UV	Urann, K., 2016, Report No ROW-0096, Appendix 2, KCP 5.1.2/02
Elendt M4 Medium (Ecotoxicology)	Primary	0.0186 mg/L	HPLC-MS/MS	Obert-Rausser, P., 2023a, Report No ROW-0155, Appendix 2, KCP 5.1.2/03
AAP Medium (Ecotoxicology)	Primary	0.0107 mg/L	HPLC-MS/MS	Obert-Rausser, P., 2023b, Report No ROW-0154, Appendix 2, KCP 5.1.2/04
50% (w/v) aqueous sucrose solution (Ecotoxicology)	Primary & Confirmatory	500 mg/L	HPLC-MS/MS	Ansaloni, T., 2023, Report No ROW-0157, Appendix 2, KCP 5.1.2/05
0.1% Triton X solution in deionised water (Ecotoxicology)	Primary & Confirmatory	5000 mg/L	HPLC-MS/MS	Ansaloni, T., 2023, Report No ROW-0157, Appendix 2, KCP 5.1.2/05
Acetone /Tween 80 (4/1) (Ecotoxicology)	Primary & Confirmatory	10 g/L	HPLC-MS/MS	Aguilar-Alberola, J. A., 2022, Report No ROW-0112, Appendix 2, KCP 5.1.2/06
50% w/v aq. sucrose + 5% acetone/Tween 80 (4/1) (Ecotoxicology)	Primary & Confirmatory	0.10 g/L	HPLC-MS/MS	Aguilar-Alberola, J. A., 2022, Report No ROW-0112, Appendix 2, KCP 5.1.2/06
Acetone (Ecotoxicology)	Primary & Confirmatory	1.0 g/L	HPLC-MS/MS	Aguilar-Alberola, J. A., 2022, Report No ROW-0112, Appendix 2, KCP 5.1.2/06
Aqueous solution (Ecotoxicology)	Primary	14.6 mg/L	HPLC-UV	Noël, E., 2016, Report No ROW- 0099, Appendix 2, KCP 5.1.2/07
Sucrose solution (Ecotoxicology)	Primary	14.3 mg/L	HPLC-UV	Noël, E., 2016, Report No ROW- 0099, Appendix 2, KCP 5.1.2/07
Royal Jelly Diet (Ecotoxicology)	Primary	2 mg/kg	HPLC-MS/MS	Picard, C., 2018b, Report No ROW-0100, Appendix 2, KCP 5.1.2/08
Larval Diet (Ecotoxicology)	Primary	1 mg/kg	HPLC-MS/MS	Aguilar-Alberola, J. A., 2019, Report No ROW-0101, Appendix 2, KCP 5.1.2/09
Deionised water (Ecotoxicology)	Primary	1 mg/L	HPLC-MS/MS	Aguilar-Alberola, J. A., 2019, Report No ROW-0101, Appendix 2, KCP 5.1.2/09

Component of residue definition: mandestrobin				
Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing / EU agreed
Orange (Residues)	Primary & Confirmatory	0.01 mg/kg	HPLC-MS/MS	Linder, M., Grewe, D. & Leischow, J., 2017, Report No ROR-0286, Appendix 2, KCP 5.1.2/10
Dried beans (Residues)	Primary & Confirmatory	0.1 mg/kg	HPLC-MS/MS	
Fresh water (Ecotoxicology)	Primary	0.1 mg/L	LC-MS/MS	Roessink, I., 2019a, Report No ROW-0103, Appendix 2, KCP 5.1.2/17
Moderately hard water (Ecotoxicology)	Primary & Confirmatory	0.200 µg/L	HPLC-MS/MS	McGuinness, A.M., 2021, Report No ROA-0075, Appendix 2, KCP 5.1.2/18
Fresh water (Ecotoxicology)	Primary	0.1 mg/L	LC-MS/MS	Roessink, I., 2019d, Report No ROW-0106, Appendix 2, KCP 5.1.2/19
Fresh water (Ecotoxicology)	Primary	0.03 mg/L	LC-MS/MS	Roessink, I., 2019c, Report No ROW-0105, Appendix 2, KCP 5.1.2/20
Fresh water (Ecotoxicology)	Primary	0.03 mg/L	LC-MS/MS	Roessink, I., 2019b, Report No ROW-0104, Appendix 2, KCP 5.1.2/21
CIPAC water D (Physical and chemical properties)	Primary	0.1% v/v	HPLC-UV	Lecocq, V., 2025, Report No ROF-0025, Appendix 2, KCP 5.1.2/22

Table 5.2-4: Validated methods for the generation of pre-authorization data

Component of residue definition: Mandestrobin R-isomer (S-2167)				
Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing / EU agreed
Honey (Residues)	Primary & Confirmatory	0.005 mg/kg	HPLC-MS/MS	Antón, B., 2022, Report No ROR-0307, Appendix 2, KCP 5.1.2/01

Table 5.2-5: Validated methods for the generation of pre-authorization data

Component of residue definition: Mandestrobin S-isomer (S-2354)				
Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing / EU agreed
Honey (Residues)	Primary & Confirmatory	0.005 mg/kg	HPLC-MS/MS	Antón, B., 2022, Report No ROR-0307, Appendix 2, KCP 5.1.2/01

Table 5.2-6: Validated methods for the generation of pre-authorization data

Component of residue definition: De-Xy-S-2200				
Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing / EU agreed
Honey (Residues)	Primary & Confirmatory	0.01 mg/kg	HPLC-MS/MS	Antón, B., 2022, Report No ROR-0307, Appendix 2, KCP 5.1.2/01
Orange (Residues)	Primary & Confirmatory	0.01 mg/kg	HPLC-MS/MS	Linder, M., Grewe, D. & Leischow, J., 2017, Report No ROR-0286, Appendix 2, KCP 5.1.2/10
Dried beans (Residues)	Primary & Confirmatory	0.1 mg/kg	HPLC-MS/MS	

Table 5.2-7: Validated methods for the generation of pre-authorization data

Component of residue definition: 4-OH-S-2200				
Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing / EU agreed
Honey (Residues)	Primary & Confirmatory	0.01 mg/kg	HPLC-MS/MS	Antón, B., 2022, Report No ROR-0307, Appendix 2, KCP 5.1.2/01
Orange (Residues)	Primary & Confirmatory	0.01 mg/kg	HPLC-MS/MS	Linder, M., Grewe, D. & Leischow, J., 2017, Report No ROR-0286, Appendix 2, KCP 5.1.2/10
Dried beans (Residues)	Primary & Confirmatory	0.01 mg/kg	HPLC-MS/MS	
Dried beans (Residues)	Primary & Confirmatory	0.01 mg/kg	HPLC-MS/MS	Linder, M. & Büdel, A., 2020, Report No ROA-0063, Appendix 2, KCP 5.1.2/15

Table 5.2-8: Validated methods for the generation of pre-authorization data

Component of residue definition: 2-CH ₂ OH-S-2200				
Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing / EU agreed
Honey (Residues)	Primary & Confirmatory	0.01 mg/kg	HPLC-MS/MS	Antón, B., 2022, Report No ROR-0307, Appendix 2, KCP 5.1.2/01
Orange (Residues)	Primary & Confirmatory	0.01 mg/kg	HPLC-MS/MS	Linder, M., Grewe, D. & Leischow, J., 2017, Report No ROR-0286, Appendix 2, KCP 5.1.2/10
Dried beans (Residues)	Primary & Confirmatory	0.01 mg/kg	HPLC-MS/MS	

Component of residue definition: 2-CH ₂ OH-S-2200				
Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing / EU agreed
Dried beans (Residues)	Primary & Confirmatory	0.01 mg/kg	HPLC-MS/MS	Linder, M. & Büdel, A., 2020, Report No ROA-0063, Appendix 2, KCP 5.1.2/15

Table 5.2-9: Validated methods for the generation of pre-authorization data

Component of residue definition: 5-CH ₂ OH-S-2200				
Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing / EU agreed
Honey (Residues)	Primary & Confirmatory	0.01 mg/kg	HPLC-MS/MS	Antón, B., 2022, Report No ROR-0307, Appendix 2, KCP 5.1.2/01

Table 5.2-10: Validated methods for the generation of pre-authorization data

Component of residue definition: DX-CA-S-2200				
Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing / EU agreed
0.5% Aqueous Methyl Cellulose Suspension (Toxicology)	Primary	1.00 mg/mL	HPLC-UV	Shibuya, E., 2017, Report No ROA-0053, Appendix 2, KCP 5.1.2/16

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

5.3.1 Analysis of the plant protection product (KCP 5.2)

Analytical methods for the determination of the active substance and relevant impurities in the plant protection product are presented under point 5.2.1.

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

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

The approved residue definition in various matrices for monitoring/enforcement purposes is summarised in the following table.

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	Mandestrobin	MRL: 0.01* mg/kg (except apricots, cherries, peaches and plums)	Regulation (EU) 2021/1247
Plant, high acid content		MRL: 0.01* mg/kg (except grapes and strawberries)	Regulation (EU) 2021/1247
Plant, high protein/high starch content (dry commodities)		MRL: 0.01* mg/kg	Regulation (EU) 2021/1247
Plant, high oil content		MRL: 0.01* mg/kg	Regulation (EU) 2021/1247
Muscle	Mandestrobin	MRL: 0.01* mg/kg	Regulation (EU) 2021/1247
Milk		MRL: 0.01* mg/kg	Regulation (EU) 2021/1247
Eggs		MRL: 0.01* mg/kg	Regulation (EU) 2021/1247
Fat		MRL: 0.01* mg/kg	Regulation (EU) 2021/1247
Liver, kidney		MRL: 0.01* mg/kg	Regulation (EU) 2021/1247
Soil (Ecotoxicology)	Mandestrobin	LOQ: 0.01 mg/kg	EFSA Journal 2015;13(5):4100
Drinking water (Human toxicology)	Mandestrobin	LOQ: 0.1 µg/L	EFSA Journal 2015;13(5):4100
Surface water (Ecotoxicology)	Mandestrobin	LOQ: 0.1 µg/L	EFSA Journal 2015;13(5):4100
Air	Mandestrobin	LOQ: 10.0 µg/m ³	EFSA Journal 2015;13(5):4100
Tissue (meat or liver)	None	Not required	EFSA Journal 2015;13(5):4100
Body fluids		Not required	EFSA Journal 2015;13(5):4100

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 mandestrobin in plant matrices is given in the following tables.

Table 5.3-2: Validated methods for food and feed of plant origin

Component of residue definition: mandestrobin				
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 & Confirmatory	0.01 mg/kg	LC-MS/MS	Göcer, M., 2012, ROA-0030, EU agreed

Component of residue definition: mandestrobin				
Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing / EU agreed
	ILV	0.01 mg/kg	LC-MS/MS	Rzepka, S., 2012, ROA-0031, EU agreed
High acid content	Primary & Confirmatory	0.01 mg/kg	LC-MS/MS	Göcer, M., 2012, ROA-0030, EU agreed
	ILV	0.01 mg/kg	LC-MS/MS	Rzepka, S., 2012, ROA-0031, EU agreed
High oil content	Primary & Confirmatory	0.01 mg/kg	GC-MS	Daneva, E., Zetzsch, A., 2012, ROA-0025, EU agreed
	ILV	0.01 mg/kg	GC-MS	Toledo, F., 2012, ROA-0026, EU agreed
High protein/high starch content (dry)	Primary & Confirmatory	0.01 mg/kg	LC-MS/MS	Göcer, M., 2012, ROA-0030, EU agreed
	ILV	0.01 mg/kg	LC-MS/MS	Rzepka, S., 2012, ROA-0031, EU agreed

Table 5.3-3: Statement on extraction efficiency

	Method for products of plant origin
Required for high-water crops, available from:	According to SANTE/2017/10632, rev. 4 the extraction efficiency of the QuEChERS based extraction procedure of the monitoring method for high-water crops can be considered as being sufficiently proven because the residues of mandestrobin in the incurred residue sample of peach obtained with the QuEChERS-based approach differed by no more than 30% compared to the results obtained with the solvents of the metabolism study. Please refer to Appendix 2, KCP 5.2/01.
Not required for high oil, high acid and high starch/protein crops, because:	Mandestrobin residues < LOQ are expected in products of plant origin and no MRLs above the LOQ are established.

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

Methods in animal matrices are not required, as no residues are to be expected in these matrices and no MRLs are proposed (MRLs set at the LOQ level).

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

An overview on the acceptable methods for analysis of mandestrobin in soil is given in the following table.

Table 5.3-4: Validated methods for soil

Component of residue definition: mandestrobin			
Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing
Primary & Confirmatory	0.01 mg/kg	LC-MS/MS	Wilde, N., 2011a, ROA-0021, EU agreed

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 mandestrobin in surface and drinking water is given in the following tables. An ILV in drinking water was generated for the renewal of mandestrobin and will be assessed at EU level.

Table 5.3-5: Validated methods for water

Component of residue definition: mandestrobin				
Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing
Drinking water	Primary & Confirmatory	0.1 µg/L	LC-MS/MS	Wilde, N., 2011b, ROA-0022, EU agreed
	ILV	0.1 µg/L	LC-MS/MS	Lindner, M. & Grewe, D., 2021, ROA-0068, Appendix 2, KCA 5.2/02
Surface water	Primary & Confirmatory	0.1 µg/L	LC-MS/MS	Wilde, N., 2011b, ROA-0022, EU agreed

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

An overview on the acceptable methods for analysis of mandestrobin in air is given in the following table.

Table 5.3-6: Validated methods for air

Component of residue definition: mandestrobin			
Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing
Primary & Confirmatory	10 µg/m ³	LC-MS/MS	Wilde, N., 2011c, ROA-0023, EU agreed

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

There is no residue definition for mandestrobin in body fluids and tissues, as such no methods are required. However, a new method for the analysis of 5-COOH-S-2200 in body fluids and tissues is available. Indeed, mandestrobin is rapidly metabolised in fluids and tissues, making it not a good marker compounds for body fluids and tissues. As a result, the metabolite 5-COOH-S-2200 has been proposed given that it is present in both fluids and tissues in both males and females, making this a more suitable analyte to analyse. This new

method was generated for the renewal of mandestrobin and will be assessed at EU level, but is also presented below.

Table 5.3-7: Validated methods for body fluid and tissues

Component of residue definition: 5-COOH-S-2200				
Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing
Blood plasma	Primary & Confirmatory	0.01 mg/L	LC-MS/MS	Lindner, M., 2021, ROA-0072, Appendix 2, KCA 5.2/03
Liver	Primary & Confirmatory	0.01 mg/kg	LC-MS/MS	Lindner, M., 2021, ROA-0072, Appendix 2, KCA 5.2/03

5.3.2.8 Other studies/ information

None.

Appendix 1 Lists of data considered in support of the evaluation

List of data submitted by the applicant and relied on

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 5.1.1/01	Lecocq, V.	2023	Validation of analytical HPLC methods for the determination of active substance content and isomer ratio in a suspension concentrate (SC) containing mandestrobin Company Report No 25587 Walloon Agricultural Research Centre, CRA-W Gembloux, Belgium Sumitomo Chemical Co., Ltd. XXXX ROA-0088 GLP Unpublished	N	XXXX
KCP 5.1.1/02	Lecocq, V.	2023	Validation of analytical GC-MS method for the determination of xylene isomers and ethylbenzene content in a suspension concentrate (SC) containing mandestrobin Company Report No 25588 Walloon Agricultural Research Centre, CRA-W Gembloux, Belgium Sumitomo Chemical Co., Ltd. XXXX. ROA-0089 GLP Unpublished	N	XXXX
KCP 5.1.2/01	Antón, B.	2022	Determination of Residues of Mandestrobin and its Metabolites in Honey, after One Application of Mandestrobin 25 SC in <i>Phacelia tanacetifolia</i> under semi-field conditions, at 4 Sites in Central and Southern Europe in 2021 Company Report No S21-01066 Eurofins Trialcamp S.L.U., Alcàsser (Valencia), Spain Sumitomo Chemical Co., Ltd. XXXX ROR-0307 GLP Unpublished	N	XXXX
KCP 5.1.2/02	Urann, K.	2016	Mandestrobin (S-2200) –Life-Cycle Toxicity Test with Mysids (<i>Americamysis bahia</i>) Company Report No 13048.6921 Smithers Viscient, Massachusetts, USA XXXX. ROW-0096	N	XXXX

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		
KCP 5.1.2/03	Obert-Rausser, P.	2023a	Mandestrobin 400 g/L: Toxicity to the Water Flea <i>Daphnia magna</i> Straus under Laboratory Conditions (Acute Immobilisation Test –Static) Company Report No S22-06619 Eurofins Aquatic Ecotoxicology GmbH, Niefern-Öschelbronn, Germany Sumitomo Chemical Co., Ltd. XXXX ROW-0155 GLP Unpublished	N	XXXX
KCP 5.1.2/04	Obert-Rausser, P.	2023b	Mandestrobin 400 g/L: Toxicity to the Single Cell Green Alga <i>Pseudokirchneriella subcapitata</i> Hindák under Laboratory Conditions Company Report No S22-06618 Eurofins Aquatic Ecotoxicology GmbH, Niefern-Öschelbronn, Germany Sumitomo Chemical Co., Ltd. XXXX ROW-0154 GLP Unpublished	N	XXXX
KCP 5.1.2/05	Ansaloni, T.	2023	Mandestrobin 40SC: Acute Oral and Contact Toxicity to the Honey bees (<i>Apis mellifera</i> L.), under Laboratory Conditions Company Report No S22-07815 Eurofins Trialcamp S.L.U., Alcàsser (Valencia), Spain Sumitomo Chemical Co., Ltd. XXXX ROW-0157 GLP Unpublished	N	XXXX
KCP 5.1.2/06	Aguilar-Alberola, J. A.	2022	S-2200 (Mandestrobin) Technical Grade: Acute Oral and Contact Toxicity Test to the Bumblebee (<i>Bombus terrestris</i> L.), under Laboratory Conditions Company Report No S21-04906 Eurofins Trialcamp S.L.U., Alcàsser (Valencia), Spain Sumitomo Chemical Co., Ltd. XXXX ROW-0112 GLP Unpublished	N	XXXX

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 5.1.2/07	Noël, E.	2016	S-2200 25 SC - A laboratory study to determine the chronic oral toxicity on the adult honey bees <i>Apis mellifera</i> L. (Hymenoptera: Apidae). Company Report No 036SRFR15C01 SynTech Research France S.A.S., La Chapelle de Guinchay, France Sumitomo Chemical Co., Ltd. XXXX, ROW-0099 GLP Unpublished	N	XXXX
KCP 5.1.2/08	Picard, C.	2018b	S-2200 (Mandestrobin): Honey Bee (<i>Apis mellifera</i> L.) Larval Toxicity Test, Repeated Exposure Company Report No 12709.6460 Smithers Viscient, Massachusetts, USA Sumitomo Chemical Co., Ltd. Valent U.S.A. LLC, ROW-0100 GLP Unpublished	N	XXXX
KCP 5.1.2/09	Aguilar-Alberola, J.A.	2019	S-2200 (Mandestrobin) 25SC: Honey Bee (<i>Apis mellifera</i> L.) Larval Toxicity Test following Repeated Exposure under laboratory conditions Company Report No S18-05345 Eurofins Trialcamp S.L.U., Alcàsser (Valencia), Spain Sumitomo Chemical Co., Ltd. XXXX, ROW-0101 GLP Unpublished	N	XXXX
KCP 5.1.2/10	Linder, M., Grewe, D. & Leischow, J.	2017	Storage Stability of Residues of Mandestrobin and its Metabolites De-Xy-S-2200, 4-OH-S-2200 and 2-CH ₂ OH-S-2200 in Dried Beans and Orange Fruit Company Report No.: S15-01208 Eurofins Agroscience Services Chem GmbH, Germany Sumitomo Chemical Co., Ltd. XXXX, ROR-0286 GLP Unpublished	N	XXXX
KCP 5.1.2/15	Linder, M. & Büdel, A.	2020	Validation of an Analytical Method for the Determination of the Mandestrobin Metabolites 4-OH-S-2200 and 2-CH ₂ OH-S-2200 in Dried Beans Company Report No.: S19-21238 (SUM-1915V) Eurofins Agroscience Services Chem GmbH, Germany	N	XXXX

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
			Sumitomo Chemical Co., Ltd. XXXX. ROA-0063 GLP Unpublished		
KCP 5.1.2/16	Shibuya E.	2017	Validation of analytical method for DX-CA-S-2200 in 0.5% methylcellulose solution Company Report No.: 16008VAL Sumika Technoservice Corporation, Japan XXXX. ROA-0053 GLP Unpublished	N	XXXX
KCP 5.1.2/17	Roessink, I.	2019a	Chronic effects of the fungicide Mandestrobin to <i>Daphnia pulex</i> Company Report No ALT.IR.2018.1 Wageningen Environmental Research, The Netherlands Sumitomo Chemical Co., Ltd. XXXX. ROW-0103 GLP Unpublished	N	XXXX
KCP 5.1.2/18	McGuinness, A.M.	2021	Mandestrobin - Validation of the Analytical Method for the Determination of the Test Substance in Aqueous Solutions Company Report No 13048.7199 Smithers, Wareham, Massachusetts, USA XXXX. ROA-0075 GLP Unpublished	N	XXXX
KCP 5.1.2/19	Roessink, I.	2019d	Chronic effects of the fungicide Mandestrobin to <i>Caridina parvidentata</i> Company Report No ALT.IR.2018.7 Wageningen Environmental Research, The Netherlands Sumitomo Chemical Co., Ltd. XXXX. ROW-0106 GLP Unpublished	N	XXXX
KCP 5.1.2/20	Roessink, I.	2019c	Chronic effects of the fungicide Mandestrobin to <i>Gammarus pulex</i> Company Report No ALT.IR.2018.4	N	XXXX

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
			Wageningen Environmental Research, The Netherlands Sumitomo Chemical Co., Ltd. XXXX. ROW-0105 GLP Unpublished		
KCP 5.1.2/21	Roessink, I.	2019b	Chronic effects of the fungicide Mandestrobin to <i>Asellus aquaticus</i> Company Report No ALT.IR.2018.2 Wageningen Environmental Research, The Netherlands Sumitomo Chemical Co., Ltd. XXXX. ROW-0104 GLP Unpublished	N	XXXX
KCP 5.1.2/22	Lecocq, V.	2025	Physical and chemical properties and storage stability tests for Mandestrobin 40 SC Third Interim Report Company Report No 25610 Walloon Agricultural Research Centre, CRA-W Gembloux, Belgium XXXX. ROF-0025 GLP Unpublished	N	XXXX
KCP 5.2/01	Lindner, M. & Fiedler, S.	2022	Cross-Validation - Comparing Amounts of Mandestrobin extracted from Samples of Peach with incurred Residues using two different Solvent Systems Company Report No S21-00874 (SUM-2101V) Eurofins Agroscience Services Chem GmbH, Germany Sumitomo Chemical Co., Ltd. XXXX. ROA-0085 GLP Unpublished	N	XXXX
KCA 5.2/02	Lindner, M. & Grewe, D.	2021	Independent Laboratory Validation of an Analytical Method for Determination of Mandestrobin in Drinking Water Company Report No.: S21-00875 (SUM-2102V) Eurofins Agroscience Services Chem GmbH, Germany XXXX. ROA-0068 GLP Unpublished	N	XXXX

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCA 5.2/03	Lindner, M.	2021	Validation of an Analytical Method for the Determination of 5-COOH-S-2200 in Blood Plasma and Liver (with amendment No. 1) Company Report No.: S21-00876 (SUM-2103V) Eurofins Agrosience Services Chem GmbH, Germany XXXX. ROA-0072 GLP Unpublished	N	XXXX

* XXXX).

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
KCP 5.1.2/11	Schernikau, N.	2010	Validation of a Method Based on Multi-Method DFG S19 for the Determination of Residues of S-2200 in High-Water and Dry Crops Company Report No.: S10-01948 Eurofins Dr. Specht GLP GmbH, Germany XXXXX. ROA-0007 GLP Unpublished	N	XXXX
KCP 5.1.2/12	Daneva, E., Breyer, N. & Taeufer, A.	2011a	Validation of an Analytical Method for Determination of S-2200 Metabolite, De-Xy-S-2200, in Seeds of Oilseed Rape, Barley (Grain and Straw) and Lettuce (Head) Company Report No.: S10-02910 Eurofins Dr. Specht GLP GmbH, Germany XXXX. ROA-0010 GLP Unpublished	N	XXXX
KCP	Daneva, E. &	2011a	Validation of an Analytical Method for Determination of S-2200 Metabolites, 4-OH-S-2200 and its	N	XXXX

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
5.1.2/13	Taeufer, A.		Conjugates, in Seeds of Oilseed Rape, Barley (Grain and Straw) and Lettuce (Head) Company Report No.: S10-02908 Eurofins Dr. Specht GLP GmbH, Germany XXXXX. ROA-0011 GLP Unpublished		
KCP 5.1.2/14	Daneva, E., Breyer, N. & Taeufer, A.	2011b	Validation of an Analytical Method for Determination of S-2200 Metabolites, 2-CH ₂ OH-S-2200 and its Conjugates, in Seeds of Oilseed Rape, Barley (Grain and Straw) and Lettuce (Head) Company Report No.: S10-02909 Eurofins Dr. Specht GLP GmbH, Germany XXXX. ROA-0012 GLP Unpublished	N	XXXX
KCP 5.2	Göcer, M.	2012	Validation of an Analytical Method for the Determination of S-2200 in Various Crop Types for Post-Registration Control and Monitoring Purpose Company Report No: P 2721 G PTRL Europe XXXX. Report ROA-0030 GLP Unpublished	N	XXXX
KCP 5.2	Rzepka, S.	2012	Independent Laboratory Validation of an Analytical Method for the Determination of S-2200 in Peach, Grape and Barley Grain for Post Registration Control and Monitoring Purpose Company Report No: SCA-1203V Eurofins Dr. Specht Laboratorien GmbH XXXX. Report ROA-0031 GLP Unpublished	N	XXXX

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	Daneva, E., Zetzsch, A.	2012	Validation of Modular Multiple Analytical Method DFG S19 (Extended and Revised Version) for the Determination of Residues of S-2200 in Seeds of Oilseed Rape Company Report No: S10-00846 Eurofins Agrosience Services Chem GmbH (EAS Chm) XXXX. Report ROA-0025 GLP Unpublished	N	XXXX
KCP 5.2	Toledo, F.	2012	Independent Laboratory Validation of Modular Multiple Analytical Method DFG S19 (Extended and Revised Version) for Determination of Residues of S-2200 in Seed of Oilseed Rape Company Report No: IF-12/02208364 SGS Institut Fresenius GmbH XXXX. Report ROA-0026 GLP Unpublished	N	XXXX
KCP 5.2	Wilde, N.	2011a	Validation of an Analytical Method for the Determination of S-2200 in Soil for Post-Registration Control and Monitoring Purpose Company Report No: P 2375 G PTRL Europe XXXX. Report ROA-0021 GLP Unpublished	N	XXXX
KCP 5.2	Wilde, N.	2011b	Validation of an Analytical Method for the Determination of S-2200 in Surface Water for Post Registration Control and Monitoring Purpose Company Report No: P 2376 G PTRL Europe XXXX. Report ROA-0022 GLP Unpublished	N	XXXX

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	Wilde, N.	2011c	Validation of an Analytical Method for the Determination of S-2200 in Air for Post Registration Control and Monitoring Purpose Company Report No: P 2377 G PTRL Europe XXXX. Report ROA-0023 GLP Unpublished	N	XXXX

* XXXX).

Appendix 2 Detailed evaluation of submitted analytical methods

A 2.1 Analytical methods for mandestrobin

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

A 2.1.1.1.1 Analytical method 1

A 2.1.1.1.1.1 Method validation

Comments of zRMS:	The method validation has been accepted. See also B7.
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Reference:	KCP 5.1.2/01 (cross reference to KCA 6.1 and 6.10.1)
Report	Determination of Residues of Mandestrobin and its Metabolites in Honey, after One Application of Mandestrobin 25 SC in <i>Phacelia tanacetifolia</i> under semi-field conditions, at 4 Sites in Central and Southern Europe in 2021, Antón, B., 2022, Report No.: S21-01066, Document No.: ROR-0307
Guideline(s):	SANTE/2020/12830 Rev.1.
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

Mandestrobin R- and S-isomers, De- Xy-S-2200: Homogenised honey samples (2.5 g) are added to 50 mL centrifuge tubes, sodium ascorbate solution (0.5 mL, 1 M) and water (25 mL, at 40°C) are added and the honey is dissolved using an ultrasonic bath. The solution is transferred to a 50 mL volumetric flask, rinsing the centrifuge tube with water (2 x 10 mL) and adding to the flask. The sample is made up to a total volume of 50 mL with water. An aliquot is filtered through a syringe filter (cellulose) and taken for analysis.

4-OH-S-2200, 2-CH₂OH-S-2200, 5-CH₂OH-S-2200: Homogenised honey samples (2.0 g) are added to 50 mL centrifuge tubes, sodium ascorbate solution (0.4 mL, 1 M) and water (5 mL, at 40°C) are added and the honey is dissolved using a linear shaker or vortex mixer and ultrasonic bath. The pH is adjusted to ~11 with sodium hydroxide solution (0.1 M) and samples are allowed to stand for 1 hour at room temperature. The pH is adjusted to 7 with hydrochloric acid (0.1 M) and sodium acetate/acetic acid buffer (10 mL, pH 5, 10 mM) is added. A β-glucosidase solution (4 mL, 10 mg/mL for enzyme activity >6 U/mg, for enzyme activities <6 U/mg the concentration is adjusted). Samples are allowed to incubate for 3 hours in a linear shaking water bath (set at 37°C). The supernatant is transferred to a 50 mL volumetric flask, rinsing the reaction tube with water/methanol (1/1, v/v, 2 x 5 mL) and adding to the flask. The sample is made up to a total volume of 50 mL with water/methanol (1/1, v/v). An aliquot is taken for analysis.

Samples are analysed by high performance liquid chromatography with tandem mass specific detection (HPLC-MS/MS) in positive electrospray ionisation mode, using a CHIRALPAK AD-RH column (150 mm x 2.1 mm, 5 µm) for the mandestrobin R- and S-isomers and an Inertsil ODS-3 column (100 mm x 2.1 mm, 3 µm) for De- Xy-S-2200, 4-OH-S-2200, 2-CH₂OH-S-2200 and 5-CH₂OH-S-2200, and gradient elution with a mobile phase of 0.1% formic acid in acetonitrile and 0.1% formic acid in water. Quantification was

performed using external calibration standards. For the mandestrobin R- and S-isomers the mass transitions m/z 314 > 192 and m/z 314 > 132 were used for quantification and confirmation, respectively. For De- Xy-S-2200 the mass transitions m/z 210 > 119 and m/z 210 > 192 were used for quantification and confirmation, respectively. For 4-OH-S-2200 the mass transitions m/z 330 > 192 and m/z 330 > 160 were used for quantification and confirmation, respectively. For 2-CH₂OH-S-2200 and 5-CH₂OH-S-2200 the mass transitions m/z 312 > 192 and m/z 312 > 119 were used for quantification and confirmation, respectively.

Results and discussions

Table A 1: Recovery results from method validation of mandestrobin R-isomer, mandestrobin S-isomer, De-Xy-S-2200, 4-OH-S-2200, 2-CH₂OH-S-2200 and 5-CH₂OH-S-2200 using the analytical method

Matrix	Analyte	Ion Transition (m/z)	Fortification Level (mg/kg)	Recoveries (%)	Mean Recovery (%)	RSD (%)
Honey	Mandestrobin R-isomer (S-2167)	314 > 192	0.005	93, 93, 94, 91, 90	92	1.8
			0.05	86, 88, 83, 89, 89	87	2.7
		314 > 132	0.005	89, 99, 93, 91, 88	92	4.5
			0.05	86, 87, 83, 88, 87	86	2.1
	Mandestrobin S-isomer (S-2354)	314 > 192	0.005	92, 87, 90, 91, 89	90	2.0
			0.05	83, 87, 84, 89, 87	86	3.3
		314 > 132	0.005	89, 89, 86, 92, 88	89	2.7
			0.05	83, 87, 83, 89, 87	86	3.3
	De-Xy-S-2200	210 > 119	0.01	92, 92, 100, 95, 104	97	5.6
			0.1	101, 101, 96, 98, 96	98	2.4
		210 > 192	0.01	100, 100, 99, 102, 100	101	1.0
			0.1	100, 102, 102, 99, 95	100	2.9
	4-OH-S-2200	330 > 192	0.01	105, 107, 104, 109, 107	107	2.1
			0.1	112, 109, 98, 99, 94	102	7.7
		330 > 160	0.01	102, 106, 105, 106, 110	106	2.7
			0.1	116, 112, 97, 95, 95	103	10
	2-CH ₂ OH-S-2200	312 > 192	0.01	84, 93, 94, 96, 101	94	6.5
			0.1	103, 95, 79, 82, 83	88	12
		312 > 119	0.01	81, 91, 84, 92, 100	89	8.6
			0.1	95, 89, 70, 82, 77	83	12
	5-CH ₂ OH-S-2200	312 > 192	0.01	80, 88, 89, 83, 92	86	5.9
			0.1	92, 78, 70, 71, 70	76	13
		312 > 119	0.01	92, 90, 92, 91, 103	94	5.7
			0.1	94, 82, 74, 75, 79	81	10

Table A 2: Characteristics for the analytical method used for validation of mandestrobin R-isomer, mandestrobin S-isomer and De-Xy-S-2200 residues in honey

	Mandestrobin R-isomer (S-2167)	Mandestrobin S-isomer (S-2354)	De-Xy-S-2200
Specificity	No residues > 30% of the LOQ were present in untreated samples. Analyte identity was confirmed by comparing the retention time of the analyte with that of standard reference material. Two ion transitions were monitored by mass spectrometry.	No residues > 30% of the LOQ were present in untreated samples. Analyte identity was confirmed by comparing the retention time of the analyte with that of standard reference material. Two ion transitions were monitored by mass spectrometry.	No residues > 30% of the LOQ were present in untreated samples. Analyte identity was confirmed by comparing the retention time of the analyte with that of standard reference material. Two ion transitions were monitored by mass spectrometry.
Calibration (type, number of data points)	m/z 314 \rightarrow 192 $r^2=0.9997$ Slope=281780 Intercept=1654 n=8 m/z 314 \rightarrow 132 $r^2=0.9997$ Slope=94897 Intercept=1025 n=8	m/z 314 \rightarrow 192 $r^2=0.9997$ Slope=280488 Intercept=3770 n=8 m/z 314 \rightarrow 132 $r^2=0.9997$ Slope=93754 Intercept=1545 n=8	m/z 210 \rightarrow 119 $r^2=0.9998$ Slope=27586 Intercept=1258 n=8 m/z 210 \rightarrow 192 $r^2=0.9998$ Slope=228141 Intercept=4028 n=8
Calibration range	Range: 0.050 – 5.0 ng/mL	Range: 0.050 – 5.0 ng/mL	Range: 0.10 – 10 ng/mL
Assessment of matrix effects	Matrix effects were found to be insignificant (i.e. $<\pm 20\%$). Matrix matched calibration standards were used throughout.	Matrix effects were found to be insignificant (i.e. $<\pm 20\%$). Matrix matched calibration standards were used throughout.	Matrix effects were found to be significant (i.e. $>\pm 20\%$). Matrix matched calibration standards were used throughout.
Assessment of extract and standard stability	Final extracts of honey were found to be stable for at least 12 days when stored under dark refrigerated (1-10°C) conditions. Stock and calibration solutions were found to be stable for at least 177 days and 13 days, respectively, when stored under dark refrigerated (1-10°C) conditions.	Final extracts of honey were found to be stable for at least 12 days when stored under dark refrigerated (1-10°C) conditions. Stock and calibration solutions were found to be stable for at least 177 days and 13 days, respectively, when stored under dark refrigerated (1-10°C) conditions.	Final extracts of honey were found to be stable for at least 12 days when stored under dark refrigerated (1-10°C) conditions. Stock and calibration solutions were found to be stable for at least 199 days and 13 days, respectively, when stored under dark refrigerated (1-10°C) conditions.
Extraction efficiency	Residues of these analytes are expected to be \leq their respective LOQs (i.e. ≤ 0.01 mg/kg). As a result of this, and in accordance with SANTE/2017/10632 rev.5, Figure 3, extraction efficiency is not required.		
Limit of detection (LOD)	0.050 ng/mL	0.050 ng/mL	0.10 ng/mL

	Mandestrobin R-isomer (S-2167)	Mandestrobin S-isomer (S-2354)	De-Xy-S-2200
Limit of quantification (LOQ)	0.005 mg/kg	0.005 mg/kg	0.01 mg/kg

Table A 3: Characteristics for the analytical method used for validation of 4-OH-S-2200, 2-CH₂OH-S-2200 and 5-CH₂OH-S-2200 residues in honey

	4-OH-S-2200	2-CH₂OH-S-2200	5-CH₂OH-S-2200
Specificity	No residues > 30% of the LOQ were present in untreated samples. Analyte identity was confirmed by comparing the retention time of the analyte with that of standard reference material. Two ion transitions were monitored by mass spectrometry.	No residues > 30% of the LOQ were present in untreated samples. Analyte identity was confirmed by comparing the retention time of the analyte with that of standard reference material. Two ion transitions were monitored by mass spectrometry.	No residues > 30% of the LOQ were present in untreated samples. Analyte identity was confirmed by comparing the retention time of the analyte with that of standard reference material. Two ion transitions were monitored by mass spectrometry.
Calibration (type, number of data points)	m/z 330 → 192 $r^2=0.9961$ Slope=465931 Intercept=26937 n=8 m/z 330 → 160 $r^2=0.9960$ Slope=169780 Intercept=10677 n=8	m/z 312 → 192 $r^2=0.9951$ Slope=148324 Intercept=9562 n=8 m/z 312 → 119 $r^2=0.9972$ Slope=41941 Intercept=1990 n=8	m/z 312 → 192 $r^2=0.9955$ Slope=88207 Intercept=5214 n=8 m/z 312 → 119 $r^2=0.9950$ Slope=29844 Intercept=1707 n=8
Calibration range	Range: 0.080 – 8.0 ng/mL	Range: 0.080 – 8.0 ng/mL	Range: 0.080 – 8.0 ng/mL
Assessment of matrix effects	Matrix effects were found to be insignificant (i.e. <±20%). Matrix matched calibration standards were used throughout.	Matrix effects were found to be insignificant (i.e. <±20%). Matrix matched calibration standards were used throughout.	Matrix effects were found to be insignificant (i.e. <±20%). Matrix matched calibration standards were used throughout.
Assessment of extract and standard stability	Final extracts of honey were found to be stable for at least 27 days when stored under dark refrigerated (1-10°C) conditions. Stock and calibration solutions were found to be stable for at least 177 days and 13 days, respectively, when stored under dark refrigerated (1-10°C) conditions.	Final extracts of honey were found to be stable for at least 27 days when stored under dark refrigerated (1-10°C) conditions. Stock and calibration solutions were found to be stable for at least 177 days and 13 days, respectively, when stored under dark refrigerated (1-10°C) conditions.	Final extracts of honey were found to be stable for at least 27 days when stored under dark refrigerated (1-10°C) conditions. Stock and calibration solutions were found to be stable for at least 177 days and 13 days, respectively, when stored under dark refrigerated (1-10°C) conditions.

	4-OH-S-2200	2-CH ₂ OH-S-2200	5-CH ₂ OH-S-2200
Extraction efficiency	Residues of these analytes are expected to be ≤ their respective LOQs (i.e. ≤ 0.01 mg/kg). As a result of this, and in accordance with SANTE/2017/10632 rev.5, Figure 3, extraction efficiency is not required.		
Limit of detection (LOD)	0.080 ng/mL	0.080 ng/mL	0.080 ng/mL
Limit of quantification (LOQ)	0.01 mg/kg	0.01 mg/kg	0.01 mg/kg

Conclusion

The analytical procedure has been successfully validated in terms of specificity, linearity, accuracy, precision, matrix effects, extract and standard stability, LOD and LOQ. The analytical procedure has been successfully validated in accordance with all of the requirements of SANTE/2020/12830, Rev.2 for the determination of mandestrobin R-isomer, mandestrobin S-isomer, De-Xy-S-2200, 4-OH-S-2200, 2-CH₂OH-S-2200 and 5-CH₂OH-S-2200 in honey.

A 2.1.1.1.2 Analytical method 2

A 2.1.1.1.2.1 Method validation

Comments of zRMS:	The method validation has been accepted. The method can be considered fit for purpose.
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Reference:	KCP 5.1.2/02 (cross reference to KCP 10.2.1/01)
Report	Mandestrobin (S-2200) –Life-Cycle Toxicity Test with Mysids (<i>Americamysis bahia</i>), Urann, K., 2016, Report No.: 13048.6921, Document No.: ROW-0096
Guideline(s):	SANCO/3029/99 rev.4.
Deviations:	No assessment of matrix effects or extract and standard stability is presented
GLP:	Yes
Acceptability:	Yes

Materials and methods

If necessary, freshwater samples are diluted with purified reagent water to fall within the calibration range. Seawater samples require no further dilution. Samples are analysed by high performance liquid chromatography with ultraviolet detection (HPLC-UV) at 215 nm, using a Chiralcel AD-RH column (150 mm x 4.6 mm, 5 µm) and isocratic elution with a mobile phase of water/acetonitrile (50:50, v/v). Calibration is performed using external standards.

Results and discussions

Table A 4: Recovery results from method validation of mandestrobin using the analytical method

Matrix	Analyte	Fortification Level (mg/L)	Recoveries (%)	Mean Recovery (%)	RSD (%)
Fresh water	Mandestrobin	0.0500	92.7, 90.8, 89.9, 92.6, 91.0	91.4	1.36

Matrix	Analyte	Fortification Level (mg/L)	Recoveries (%)	Mean Recovery (%)	RSD (%)
		100	98.7, 96.5, 98.3, 95.0, 96.6	97.0	1.54
Seawater	Mandestrobin	0.00500	95.5, 100, 97.0, 96.8, 93.8	96.6	2.36
		0.0100	102, 101, 105, 101, 104	103	1.75

Table A 5: Characteristics for the analytical method used for validation of mandestrobin residues in fresh water and seawater

	Mandestrobin
Specificity	No residues > 30% of the LOQ were present in untreated samples. Analyte identity was confirmed by comparing the retention time of the analyte with that of standard reference material.
Calibration (type, number of data points)	Fresh water Mandestrobin R-isomer: $r^2 = 0.99669$ Slope=3577.749 Intercept=-4.6704 n=6 Mandestrobin S-isomer: $r^2 = 0.99349$ Slope=3452.550 Intercept=-4.5981 n=6 Seawater Mandestrobin R-isomer: $r^2 = 0.99937$ Slope=3325.878 Intercept=0.1263 n=6 Mandestrobin S-isomer: $r^2 = 0.99799$ Slope=3104.694 Intercept=1.6360 n=6
Calibration range	Range: 0.004 – 0.1 mg/L (0.002 – 0.05 mg/L as individual R- and S-isomers)
Assessment of matrix effects	Not assessed
Assessment of extract and standard stability	Not assessed
Limit of detection (LOD)	0.004 mg/L (0.002 mg/L for individual R- and S-isomers)
Limit of quantification (LOQ)	Fresh water: 0.0500 mg/L Seawater: 0.00500 mg/L

Conclusion

The analytical procedure has been successfully validated in terms of specificity, linearity, accuracy, precision, LOD and LOQ. Although not in accordance with all the requirements of SANTE/2020/12830, Rev.2, as matrix effects and extract and standard stability have not been assessed, the acceptable recovery and precision values demonstrate this method is capable of determining mandestrobin in fresh water and seawater and thus the method can be considered fit for purpose. Additionally, the method satisfies the mini-

num requirements of an existing method under SANTE/2020/12830, Rev.2, so can be considered acceptable.

A 2.1.1.1.3 Analytical method 3

A 2.1.1.1.3.1 Method validation

Comments of zRMS:	The method validation has been accepted. The analyte samples was determined with HPLC-MS/MS. The analytical method was fully validated in accordance with the requirements.
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Reference:	KCP 5.1.2/03 (cross reference to KCP 10.2.1/02)
Report	Mandestrobin 400 g/L: Toxicity to the Water Flea <i>Daphnia magna</i> Straus under Laboratory Conditions (Acute Immobilisation Test –Static) and Report Amendment 1, Obert-Rausser, P., 2023a, Report No.: S22-06619, Document No.: ROW-0155
Guideline(s):	SANTE/2020/12830 Rev.1.
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

Thawed samples of Elendt M4 Medium (10 mL) are mixed with acetonitrile (10 mL) and shaken well using a vortex mixer. If necessary, samples are further diluted with acetonitrile/test medium (1/1, v/v) to fall within the calibration range. Samples are analysed by high performance liquid chromatography with tandem mass specific detection (HPLC-MS/MS) in positive electrospray ionisation mode, using an Agilent Zorbax Eclipse XDB-C18 column (50 mm x 4.6 mm, 1.8 µm) fitted with a C18 UHPLC guard column (2.1 mm) and gradient elution with a mobile phase of 0.5% formic acid in water and acetonitrile. Quantification is performed using external calibration standards monitoring the mass transitions m/z 314 > 192 and m/z 314 > 160 for quantification and confirmation, respectively.

Results and discussions

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

Matrix	Analyte	Ion Transition (m/z)	Fortification Level (mg/L)	Recoveries (%)	Mean Recovery (%)	RSD (%)
Elendt M4 Medium	Mandestrobin	314 > 192	0.0186	94, 86, 83, 103, 105	94	10
			3.88	96, 92, 95, 94, 90	93	3

Table A 7: Characteristics for the analytical method used for validation of mandestrobin residues in Elendt M4 Medium

	Mandestrobin
Specificity	No residues > 30% of the LOQ were present in untreated samples. Analyte identity was confirmed by comparing the retention time of the analyte with that of standard reference material. Two ion transitions

	Mandestrobin
	were monitored by mass spectrometry.
Calibration (type, number of data points)	m/z 314 \rightarrow 192 $r = 0.9998$ Slope= 3.4×10^6 Intercept= 2.32×10^4 $n=10$
Calibration range	Range: 0.0130 – 1.30 ng/mL (0.00520 – 0.520 mg/L)
Assessment of matrix effects	Matrix effects were found to be significant (i.e. $>\pm 20\%$) at some concentrations. Matrix matched calibration standards were used throughout.
Assessment of extract and standard stability	Final extracts of Elendt M4 Medium were found to be stable for at least 5 days when stored under dark refrigerated (1-10°C) conditions. The stock solution was found to be stable for at least 45 days when stored under dark refrigerated (1-10°C) conditions.
Limit of detection (LOD)	0.00520 mg/L
Limit of quantification (LOQ)	0.0186 mg/L

Conclusion

The analytical procedure has been successfully validated in terms of specificity, linearity, accuracy, precision, matrix effects, extract and standard stability, LOD and LOQ. The analytical procedure has been successfully validated in accordance with all of the requirements of SANTE/2020/12830, Rev.2 for the determination of mandestrobin in Elendt M4 Medium.

A 2.1.1.1.4 Analytical method 4

A 2.1.1.1.4.1 Method validation

Comments of zRMS:	The method validation has been accepted. The content of the analyte in the test solution samples was determined by analysing with HPLC-MS/MS.
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Reference:	KCP 5.1.2/04 (cross reference to KCP 10.2.1/03)
Report	Mandestrobin 400 g/L: Toxicity to the Single Cell Green Alga <i>Pseudokirchneriella subcapitata</i> Hindák under Laboratory Conditions and Report Amendment 1, Obert-Rausser, P., 2023b, Report No.: S22-06618, Document No.: ROW-0154
Guideline(s):	SANTE/2020/12830 Rev.1.
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

Thawed samples of AAP Medium (10 mL) are mixed with acetonitrile (10 mL) and shaken well using a vortex mixer. If necessary, samples are further diluted with acetonitrile/test medium (1/1, v/v) to fall within the calibration range. Samples are analysed by high performance liquid chromatography with tandem mass

specific detection (HPLC-MS/MS) in positive electrospray ionisation mode, using an Agilent Zorbax Eclipse XDB-C18 column (50 mm x 4.6 mm, 1.8 µm) fitted with a C18 UHPLC guard column (2.1 mm) and gradient elution with a mobile phase of 0.5% formic acid in water and acetonitrile. Quantification is performed using external calibration standards monitoring the mass transitions m/z 314 > 192 and m/z 314 > 160 for quantification and confirmation, respectively.

Results and discussions

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

Matrix	Analyte	Ion Transition (m/z)	Fortification Level (mg/L)	Recoveries (%)	Mean Recovery (%)	RSD (%)
AAP Medium	Mandestrobin	314 > 192	0.0107	111, 94, 97, 97, 94	99	7
			14.5	102, 105, 103, 103, 108	104	2

Table A 9: Characteristics for the analytical method used for validation of mandestrobin residues in AAP Medium

	Mandestrobin
Specificity	No residues > 30% of the LOQ were present in untreated samples. Analyte identity was confirmed by comparing the retention time of the analyte with that of standard reference material. Two ion transitions were monitored by mass spectrometry.
Calibration (type, number of data points)	m/z 314 → 192 $r = 0.9999$ Slope= 3.39×10^6 Intercept= 1.27×10^4 $n=10$
Calibration range	Range: 0.0160 – 1.60 ng/mL (0.00320 – 0.320 mg/L)
Assessment of matrix effects	Matrix effects were found to be insignificant (i.e. $\leq \pm 20\%$). Matrix matched calibration standards were used throughout.
Assessment of extract and standard stability	Final extracts of AAP Medium were found to be stable for at least 10 days when stored for 9 days frozen followed by 1 day under dark refrigerated (1-10°C) conditions. The stock solution was found to be stable for at least 45 days when stored under dark refrigerated (1-10°C) conditions.
Limit of detection (LOD)	0.00320 mg/L
Limit of quantification (LOQ)	0.0107 mg/L

Conclusion

The analytical procedure has been successfully validated in terms of specificity, linearity, accuracy, precision, matrix effects, extract and standard stability, LOD and LOQ. The analytical procedure has been successfully validated in accordance with all of the requirements of SANTE/2020/12830, Rev.2 for the determination of mandestrobin in AAP Medium.

A 2.1.1.1.5 Analytical method 5

A 2.1.1.1.5.1 Method validation

Comments of zRMS:	<p>The method validation has been accepted.</p> <p>The objective was to analyse aliquots of three dilutions of Mandestrobin 40SC prepared in 50 % (w/v) sucrose solution for acute oral toxicity testing and three dilutions in 0.1 % (v/v) aqueous Triton X solution for levels of mandestrobin.</p> <p>The nominal limit of quantification (LOQ) of the analytical method was 500 mg/L for 50 % (w/v) sucrose solution and 5000 mg/L for 0.1 % (v/v) aqueous Triton X solution. Two mass transitions were evaluated. One is proposed as quantification transition but both selected mass transitions proved to be interchangeably applicable for quantification and confirmation.</p> <p>All mean values at fortification levels of LOQ and higher level for two mass transitions are within 70 % - 120 % with relative standard deviations ≤ 20 %.</p>
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Reference:	KCP 5.1.2/05 (cross reference to KCP 10.3.1.1.1/01 & KCP 10.3.1.1.2/01)
Report	Mandestrobin 40SC: Acute Oral and Contact Toxicity to the Honey bees (<i>Apis mellifera</i> L.), under Laboratory Conditions, Ansaloni, T., 2023, Report No.: S22-07815, Document No.: ROW-0157
Guideline(s):	SANTE/2020/12830 Rev.2.
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

Samples of 50% (w/v) aqueous sucrose solution (100 µL) are diluted with acetone/water (7:3, v/v, volume dependent on concentration level) and thoroughly mixed. An aliquot (100 µL) is then diluted with water/acetonitrile (7:3, v/v, volume depending on concentration level).

Samples of 0.1% Triton X solution in deionised water (100 µL) are diluted with acetone (volume dependent on concentration level) and thoroughly mixed. An aliquot is then further diluted with water/acetonitrile (7:3, v/v, volume depending on concentration level).

Samples are analysed by high performance liquid chromatography with tandem mass specific detection (HPLC-MS/MS) in positive electrospray ionisation mode, using an Supelco Ascentis Express C18 column (50 mm x 2.1 mm, 2.7 µm) fitted with a Phenomenex SecurityGuard™ ULTRA for C18 UHPLC pre-column (2.1 mm) and gradient elution with a mobile phase of 0.1% formic acid in acetonitrile and 0.1% formic acid in water. Quantification is performed using external calibration standards monitoring the mass transitions m/z 314 > 192 and m/z 314 > 160 for quantification and confirmation, respectively.

Results and discussions

Table A 10: Recovery results from method validation of mandestrobin using the analytical method

Matrix	Analyte	Ion Transition (m/z)	Fortification Level (mg/L)	Recoveries (%)	Mean Recovery (%)	RSD (%)
50% (w/v) aqueous sucrose solution	Mandestrobin	314 > 192	500	107, 114, 131, 119, 102	114	9.7
			26000	95, 99, 98, 97, 92	96	2.8
		314 > 160	500	105, 112, 132, 118, 103	114	10

Matrix	Analyte	Ion Transition (m/z)	Fortification Level (mg/L)	Recoveries (%)	Mean Recovery (%)	RSD (%)
			26000	93, 102, 95, 92, 94	95	4.2
0.1% Triton X solution in deionised water	Mandestrobin	314 > 192	5000	104, 112, 100, 97, 95	102	6.5
			260000	91, 102, 106, 106, 90	99	8.0
		314 > 160	5000	102, 112, 101, 96, 96	102	6.4
			260000	91, 101, 105, 106, 88	98	6.4

Table A 11: Characteristics for the analytical method used for validation of mandestrobin residues in 50% (w/v) aqueous sucrose solution and 0.1% Triton X solution in deionised water

	Mandestrobin
Specificity	No residues > 30% of the LOQ were present in untreated samples or the reagent blanks. Analyte identity was confirmed by comparing the retention time of the analyte with that of standard reference material. Two ion transitions were monitored by mass spectrometry.
Calibration (type, number of data points)	<i>m/z</i> 314 → 192 r = 1.0000 Slope=9687.9817 Intercept=606.1615 n=6 <i>m/z</i> 314 → 160 r = 1.0000 Slope=9635.9174 Intercept=566.0634 n=6
Calibration range	Range: 0.60 – 20 ng/mL (60 – 2000 mg/L)
Assessment of matrix effects	Matrix effects were found to be insignificant (i.e. <±20%). Solvent-based calibration standards were used throughout.
Assessment of extract and standard stability	Final extracts were found to be stable for at least 11 days when stored under dark refrigerated (1-10°C) conditions. The stock solution was found to be stable for at least 155 days when stored under refrigerated (1-10°C) conditions. Calibration solutions were found to be stable for at least 26 days when stored under refrigerated (1-10°C) conditions. See KCP 5.1.2/09 below.
Limit of detection (LOD)	60 mg/L
Limit of quantification (LOQ)	50% (w/v) aqueous sucrose solution: 500 mg/L 0.1% Triton X solution in deionised water: 5000 mg/L

Conclusion

The analytical procedure has been successfully validated in terms of specificity, linearity, accuracy, precision, matrix effects, extract and standard stability, LOD and LOQ. The analytical procedure has been successfully validated in accordance with all of the requirements of SANTE/2020/12830, Rev.2 for the determination of mandestrobin in 50% (w/v) aqueous sucrose solution and 0.1% Triton X solution in deionised water.

A 2.1.1.1.6 Analytical method 6

A 2.1.1.1.6.1 Method validation

Comments of zRMS:	The method validation has been accepted. Analytical data were required by the guidelines to verify the actual concentration of the test item (representative sample) and its solubility in the solvent. Quantification was performed by liquid chromatography with tandem mass spectrometry (LC-MS/MS).
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Reference:	KCP 5.1.2/06 (cross reference to KCP 10.3.1.1.1/02 & KCP 10.3.1.1.2/02)
Report	S-2200 (Mandestrobin) Technical Grade: Acute Oral and Contact Toxicity Test to the Bumblebee (<i>Bombus terrestris</i> L.), under Laboratory Conditions, Aguilar-Alberola, J. A., 2022, Report No.: S21-04906, Document No.: ROW-0112
Guideline(s):	SANTE/2020/12830 Rev.1.
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

Samples of oral stock, oral feeding or contact application solutions are allowed to reach room temperature before homogenization and dilution with acetone and/or water/acetonitrile (7:3, v/v) as appropriate. Samples are analysed by high performance liquid chromatography with tandem mass specific detection (HPLC-MS/MS) in positive electrospray ionization mode using an Ascentis Express C18 column (50 mm x 2.1 mm, 2.7 µm) and gradient elution with a mobile phase comprising of 0.1% formic acid in acetonitrile and 0.1% formic acid in water. Quantification is performed using external calibration standards monitoring the mass transitions m/z 314 > 192 and m/z 314 > 160 for quantification and confirmation, respectively.

Results and discussions

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

Matrix	Analyte	Ion Transition (m/z)	Fortification Level (g/L)	Recoveries (%)	Mean Recovery (%)	RSD (%)
Oral stock solution (Acetone /Tween 80 (4/1))	Mandestrobin	314 > 192	10	116, 113, 108, 113, 115	113	2.8
			100	114, 111, 105, 107, 105	108	3.7
		314 > 160	10	111, 110, 105, 111, 112	110	2.4
			100	112, 107, 105, 110, 104	108	3.3
Oral feeding solution (50% w/v aq. sucrose + 5% acetone/Tween 80 (4/1))	Mandestrobin	314 > 192	0.10	84, 82, 90, 89, 73	83	8.3
			5.0	77, 89, 90, 90, 100	89	9.0
		314 > 160	0.10	87, 85, 89, 88, 75	85	6.9
			5.0	76, 88, 92, 89, 98	89	9.3
	Mandestrobin	314 > 192	1.0	95, 95, 93, 98, 98	96	2.3

Matrix	Analyte	Ion Transition (m/z)	Fortification Level (g/L)	Recoveries (%)	Mean Recovery (%)	RSD (%)
Contact application solution (Acetone)			50	97, 95, 96, 93, 94	95	1.6
		314 > 160	1.0	96, 96, 93, 97, 96	96	1.7
			50	96, 95, 94, 94, 93	95	1.2

Table A 13: Characteristics for the analytical method used for validation of mandestrobin residues in oral stock, oral feeding or contact application solutions

	Mandestrobin
Specificity	No residues > 30% of the LOQ were present in untreated samples. Analyte identity was confirmed by comparing the retention time of the analyte with that of standard reference material. Two ion transitions were monitored by mass spectrometry.
Calibration (type, number of data points)	m/z 314 → 192 $r = 0.9999$ Slope=49797.2304 Intercept=1381.2074 $n=7$ m/z 314 → 160 $r = 0.9999$ Slope=22279.6224 Intercept=1101.7482 $n=7$
Calibration range	Range: 0.60 – 20 ng/mL
Assessment of matrix effects	Matrix effects were found to be insignificant (i.e. <±20%). Solvent-based calibration standards were used throughout.
Assessment of extract and standard stability	Final extracts of oral stock, oral feeding and contact application solutions were found to be stable for at least 24, 12 and 17 days respectively, when stored under dark refrigerated (1-10°C) conditions. The stock solution was found to be stable for at least 155 days when stored under refrigerated (1-10°C) conditions. Calibration solutions were found to be stable for at least 26 days when stored under refrigerated (1-10°C) conditions.
Limit of detection (LOD)	0.60 ng/mL
Limit of quantification (LOQ)	Oral stock: 10 g/L Oral feeding: 0.10 g/L Contact application: 1.0 g/L

Conclusion

The analytical procedure has been successfully validated in terms of specificity, linearity, accuracy, precision, matrix effects, extract and standard stability, LOD and LOQ. The analytical procedure has been successfully validated in accordance with all of the requirements of SANTE/2020/12830, Rev.2 for the determination of mandestrobin in oral stock, oral feeding or contact application solutions.

A 2.1.1.1.7 Analytical method 7

A 2.1.1.1.7.1 Method validation

Comments of zRMS:	<p>The method validation has been accepted.</p> <p>The objective of the analytical phase is to validate analytical methods for the determination of mandestrobin in treatment solutions of S-2200 25 SC in compliance with SANCO/3029/99 rev. 4 from 11/07/00 and to analyse mandestrobin in treatment solutions of S-2200 25 SC. Mandestrobin is quantified by liquid chromatography using a reverse phase column and a UV detector, following an internal method. A validation of an analytical method is performed during this study regarding the specificity, the linearity, the accuracy, the precision, the limit of detection (LOD) and the limit of quantification (LOQ) of the method. Validations in treatment aqueous solutions as well as in treatment sucrose solutions resulted with the LOQs of mandestrobin at 2.93 mg/L and at 3.00 mg/L, respectively.</p>
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Reference:	KCP 5.1.2/07 (cross reference to KCP 10.3.1.2/02)
Report	S-2200 25 SC - A laboratory study to determine the chronic oral toxicity on the adult honey bees <i>Apis mellifera</i> L. (Hymenoptera: Apidae), Noël, E., 2016, Report No.: 036SRFR15C01, Document No.: ROW-0099
Guideline(s):	SANCO/3029/99 rev.4
Deviations:	No assessment of matrix effects or extract and standard stability is presented
GLP:	Yes
Acceptability:	Yes

Materials and methods

Shaken aqueous samples (0.5 mL) are diluted with methanol (to 50 mL). An aliquot (1.3 mL) is diluted with methanol (to 10 mL).

Shaken 50% sucrose solution samples (0.7 mL) are diluted with methanol (0.7 mL) and homogenised.

Samples are analysed by high performance liquid chromatography with ultra-violet detection (HPLC-UV) at 210 nm, using a Phenomenex Synergi 4µm hydroRP80A column (25 cm x 4.6 mm, 4.0 µm) and gradient elution with a mobile phase comprising of methanol/water (8/2, v/v) and water. Calibration is performed using external standards.

Results and discussions

Table A 14: Recovery results from method validation of mandestrobin using the analytical method

Matrix	Analyte	Fortification Level (mg/L)	Recoveries (%)	Mean Recovery (%)	RSD (%)
Aqueous solution	Mandestrobin	14.6	101.7, 101.4, 101.1, 100.7, 100.6	101.1	0.46
		17.6*	100.3, 99.7, 100.1, 99.0	99.8	0.54
Sucrose solution	Mandestrobin	14.3*	99.4, 101.4, 104.8, 105.0	102.7	2.64
		17.1	104.7, 105.0, 104.6, 104.5, 104.8	104.7	0.15

*Recovery results were obtained for only four samples as there was an issue with injection.

Table A 15: Characteristics for the analytical method used for validation of mandestrobin residues in aqueous and sucrose solutions

	Mandestrobin
Specificity	No residues > 30% of the LOQ were present in untreated samples or the reagent blanks. Analyte identity was confirmed by comparing the retention time of the analyte with that of standard reference material.
Calibration (type, number of data points)	$r = 0.9990$ Slope= 1.22×10^6 Intercept= 3.26×10^4 $n=5$
Calibration range	Range: 5.86 – 18.09 mg/L
Assessment of matrix effects	Not assessed
Assessment of extract and standard stability	Not assessed
Limit of detection (LOD)	5.86 mg/L
Limit of quantification (LOQ)	Aqueous solution: 14.6 mg/L Sucrose solution: 14.3 mg/L

Conclusion

The analytical procedure has been successfully validated in terms of specificity, linearity, accuracy, precision, LOD and LOQ. Although not in accordance with all the requirements of SANTE/2020/12830, Rev.2, as matrix effects and extract and standard stability have not been assessed, the acceptable recovery and precision values demonstrate this method is capable of determining mandestrobin in aqueous and sucrose solutions and thus the method can be considered fit for purpose. Additionally, the method satisfies the minimum requirements of an existing method under SANTE/2020/12830, Rev.2, so can be considered acceptable.

A 2.1.1.1.8 Analytical method 8

A 2.1.1.1.8.1 Method validation

Comments of zRMS:	The method validation has been accepted. The method using liquid chromatography with tandem mass spectrometry detection (LC-MS/MS) was validated in royal jelly diet by dosing with S-2200 at concentrations of 2000, and 3,000,000 µg/kg. Recoveries averaged $91.9 \pm 2.79\%$, with a limit of quantitation (LOQ) of 2000 µg/kg, the lowest fortification level, and a method detection limit (MDL) of 1000 µg/kg. Acceptance criteria for mean recoveries of samples prepared during the method validation was 70.0 to 110%.
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Reference:	KCP 5.1.2/08 (cross reference to KCP 10.3.1.2/01 & KCP 10.3.1.3/01)
Report	S-2200 (Mandestrobin): Honey Bee (<i>Apis mellifera</i> L.) Larval Toxicity Test, Repeated Exposure, Picard, C., 2018b, Report No.: 12709.6460, Document No.: ROW-0100
Guideline(s):	SANCO/3029/99 rev.4
Deviations:	No assessment of matrix effects or extract and standard stability is presented
GLP:	Yes

Acceptability: Yes

Materials and methods

Royal Jelly Diet samples (2.00 g) are appropriately diluted in acetonitrile/water (50:50, v/v). Samples are analysed by high performance liquid chromatography with tandem mass specific detection (HPLC-MS/MS) in positive electrospray ionization mode using a Phenomenex Lux Cellulose-3 column (150 mm x 2.0 mm, 3 µm) and isocratic elution with a mobile phase of 0.1% formic acid in water/0.1% formic acid in acetonitrile (65:35, v/v). Quantification is performed using external calibration standards monitoring the mass transition m/z 314.3 > 192.2.

Results and discussions

Table A 16: Recovery results from method validation of mandestrobin using the analytical method

Matrix	Analyte	Fortification Level (mg/kg)	Recoveries (%)	Mean Recovery (%)	RSD (%)
Royal Jelly Diet	Mandestrobin	2	95.0, 90.2, 90.4, 92.9, 91.6	92.0	2.18
		3,000	88.2, 90.2, 90.7, 92.5, 96.9	91.7	3.56

Table A 17: Characteristics for the analytical method used for validation of mandestrobin residues in Royal Jelly Diet

	Mandestrobin
Specificity	No residues > 30% of the LOQ were present in untreated samples or the reagent blanks. Analyte identity was confirmed by comparing the retention time of the analyte with that of standard reference material.
Calibration (type, number of data points)	Mandestrobin R-isomer: $r^2 = 1.00$ Slope=404858.658 Intercept=-8700.6986 n=6 Mandestrobin S-isomer: $r^2 = 1.00$ Slope=407413.918 Intercept=-8844.2877 n=6
Calibration range	Range: 1.0 – 10.0 µg/L (0.50 – 5.0 µg/L as individual R- and S-isomers)
Assessment of matrix effects	Not assessed
Assessment of extract and standard stability	Not assessed
Limit of detection (LOD)	1.0 µg/L (0.50 µg/L as individual R- and S-isomers)
Limit of quantification (LOQ)	2 mg/kg

Conclusion

The analytical procedure has been successfully validated in terms of specificity, linearity, accuracy, precision, LOD and LOQ. Although not in accordance with all the requirements of SANTE/2020/12830, Rev.2, as matrix effects and extract and standard stability have not been assessed, the acceptable recovery and

precision values demonstrate this method is capable of determining mandestrobin in Royal Jelly Diet and thus the method can be considered fit for purpose. Additionally, the method satisfies the minimum requirements of an existing method under SANTE/2020/12830, Rev.2, so can be considered acceptable.

A 2.1.1.1.9 Analytical method 9

A 2.1.1.1.9.1 Method validation

Comments of zRMS:	<p>The method validation has been accepted.</p> <p>Quantification was performed by use of LC-MS/MS detection.</p> <p>The analytical method was validated for the determination of mandestrobin in larval diet and deionised water.</p>
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Reference:	KCP 5.1.2/09 (cross reference to KCP 10.3.1.3/02)
Report	S-2200 (Mandestrobin) 25SC: Honey bee (<i>Apis mellifera</i> L.) Larval Toxicity Test following Repeated Exposure under laboratory conditions, Aguilar-Alberola, J. A., 2019, Report No.: S18-05345, Document No.: ROW-0101
Guideline(s):	SANCO/3029/99 rev.4
Deviations:	No assessment of standard stability is presented
GLP:	Yes
Acceptability:	Yes

Materials and methods

Thawed larval diet samples are diluted with water (25 mL) and shaken well by vortex. Samples are diluted with acetonitrile (100 mL) and the samples are shaken well by hand and vortexed for at least 2 minutes. Five QuEChERS salt mixtures (each containing 4 g magnesium sulphate, 1 g sodium chloride, 1 g trisodium citrate, 0.5 g disodium citrate sesquihydrate) are added to each sample and they are shaken well for at least 15 minutes. An aliquot (40 mL) is centrifuged for 2 minutes at 4000 rpm and the final sample extracts are diluted by a factor of 20 with acetonitrile/water (1:1, v/v) then further with acetonitrile/water (1:1, v/v) to fall within the calibration range.

Thawed deionised water samples are homogenised well using a vortex mixer and diluted with acetonitrile/water (1:1, v/v) to fall within the calibration range.

Samples are analysed by high performance liquid chromatography with tandem mass specific detection (HPLC-MS/MS) in positive electrospray ionisation mode, using an Agilent ZORBAX Eclipse XDB-C18 column (50 mm x 4.6 mm, 1.8 µm) fitted with a Phenomenex UHPLC guard column with C18 cartridge (2.1 mm) and gradient elution with a mobile phase comprising of 0.1% formic acid in water and 0.1% formic acid in methanol. Quantification is performed using external calibration standards monitoring the mass transitions m/z 314 > 192 and m/z 314 > 160 for quantification and confirmation, respectively.

Results and discussions

Table A 18: Recovery results from method validation of mandestrobin using the analytical method

Matrix	Analyte	Fortification Level	Recoveries (%)	Mean Recovery (%)	RSD (%)
Larval Diet	Mandestrobin	1 mg/kg	90, 93, 93, 98, 93	93	3
		850 mg/kg	88, 87, 98, 101, 90	93	7
Deionised water	Mandestrobin	1 mg/L	96, 83, 93, 95, 98	93	6
		9800 mg/L	96, 95, 98, 100, 92	96	3

Table A 19: Characteristics for the analytical method used for validation of mandestrobin residues in larval diet and deionised water

	Mandestrobin
Specificity	No residues > 30% of the LOQ were present in untreated samples. Analyte identity was confirmed by comparing the retention time of the analyte with that of standard reference material. Two ion transitions were monitored by mass spectrometry.
Calibration (type, number of data points)	$r = 0.9984$ Slope= 3.79×10^6 Intercept= 1.48×10^5 $n=5$
Calibration range	Range: 0.3 – 5 ng/mL
Assessment of matrix effects	Matrix effects were found to be insignificant (i.e. $< \pm 20\%$). Solvent-based calibration standards were used throughout.
Assessment of extract and standard stability	Final extracts were found to be stable for at least 11 days when stored under dark refrigerated (1-10°C) conditions. The stock solution was found to be stable for at least 155 days when stored under refrigerated (1-10°C) conditions. Calibration solutions were found to be stable for at least 26 days when stored under refrigerated (1-10°C) conditions.
Limit of detection (LOD)	0.3 ng/mL
Limit of quantification (LOQ)	Larval diet: 1 mg/kg Deionised water: 1 mg/L

Conclusion

The analytical procedure has been successfully validated in terms of specificity, linearity, accuracy, precision, matrix effects, extract stability, LOD and LOQ. Although not in accordance with all the requirements of SANTE/2020/12830, Rev.2, as standard stability has not been assessed, the acceptable recovery and precision values demonstrate this method is capable of determining mandestrobin in larval diet and deionised water and thus the method can be considered fit for purpose. Additionally, the method satisfies the minimum requirements of an existing method under SANTE/2020/12830, Rev.2, so can be considered acceptable.

A 2.1.1.1.10 Analytical method 10

A 2.1.1.1.10.1 Method validation

Comments of zRMS:	<p>The method validation have been accepted for the study S15-01208. (see also B7). The studies ROA-0007, ROA-0010, ROA-0011 and ROA-0012 were not provided. It has no impact on the conclusion of the authorisation request of the applicant.</p> <p><i>The applicant comment: The studies ROA-0007, ROA-0010, ROA-0011 and ROA-0012 were evaluated and peer reviewed for mandestrobin approval, therefore it is not necessary to submit them in the frame of this dossier. A reference to the EU peer review can be added in the grey box in section A 2.1.1.10.1. These studies are also listed in Appendix 1 in the Table «List of data submitted or referred to by the applicant and relied on, but already evaluated at EU peer review».</i></p>
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Reference:	KCP 5.1.2/10 (cross reference to KCA 6.1)
Report	Storage Stability of Residues of Mandestrobin and its Metabolites De-Xy-S-2200, 4-OH-S-2200 and 2-CH ₂ OH-S-2200 in Dried Beans and Orange Fruit, Linder, M., Grewe, D. & Leischow, J., 2017, Report No.: S15-01208, Document No.: ROR-0286
Guideline(s):	SANCO/3029/99, Rev. 4
Deviations:	No assessment of extract and standard stability is presented Reduced validation not presented at the LOQ
GLP:	Yes
Acceptability:	Yes
Reference:	KCP 5.1.2/11
Report	Validation of a Method Based on Multi-Method DFG S19 for the Determination of Residues of S-2200 in High-Water and Dry Crops, Schernikau, N., 2010, Report No.: S10-01948, Document No.: ROA-0007
Guideline(s):	SANCO/3029/99, Rev. 4
Deviations:	No assessment of extract and standard stability is presented
GLP:	Yes
Acceptability:	n/a
Reference:	KCP 5.1.2/12
Report	Validation of an Analytical Method for Determination of S-2200 Metabolite, De-Xy-S-2200, in Seeds of Oilseed Rape, Barley (Grain and Straw) and Lettuce (Head), Daneva, E., Breyer, N. & Taeufer, A., 2011a, Report No.: S10-02910, Document No.: ROA-0010
Guideline(s):	SANCO/3029/99, Rev. 4
Deviations:	No assessment of extract and standard stability is presented
GLP:	Yes
Acceptability:	n/a
Reference:	KCP 5.1.2/13
Report	Validation of an Analytical Method for Determination of S-2200 Metabolites, 4-OH-S-2200 and its Conjugates, in Seeds of Oilseed Rape, Barley (Grain and Straw) and Lettuce (Head), Daneva, E. & Taeufer, A., 2011a, Report No.: S10-02908, Document No.: ROA-0011

Guideline(s):	SANCO/3029/99, Rev. 4
Deviations:	No assessment of extract and standard stability is presented
GLP:	Yes
Acceptability:	n/a
Reference:	KCP 5.1.2/14
Report	Validation of an Analytical Method for Determination of S-2200 Metabolites, 2-CH ₂ OH-S-2200 and its Conjugates, in Seeds of Oilseed Rape, Barley (Grain and Straw) and Lettuce (Head), Daneva, E., Breyer, N. & Taeufer, A., 2011b, Report No.: S10-02909, Document No.: ROA-0012
Guideline(s):	SANCO/3029/99, Rev. 4
Deviations:	No assessment of extract and standard stability is presented
GLP:	Yes
Acceptability:	n/a

Materials and methods

Mandestrobin: Homogenised samples of dried beans (5 g) and orange (10 g) are weighed into centrifuge tubes. Dried beans are soaked in water (10 mL) for 10 minutes. Both samples are shaken vigorously for 2 minutes with acetonitrile (10 mL). Magnesium sulfate (4 g), sodium chloride (1 g), trisodium citrate dihydrate (1 g) and disodium hydrogencitrate sesquihydrate (0.5 g) are added and the contents are shaken for 2 minutes by hand. The upper acetonitrile phase is isolated and frozen for ~3 hours at ≤18°C, the samples are centrifuged for 2 minutes at about 4000 rpm while still cold. PSA (40 mg) and magnesium sulfate (225 mg) are weighed into a safe-lock tube, to which an aliquot (~1.5 mL) of the supernatant is added. The tube is shaken vigorously by vortex mixer, by hand for 30 seconds and centrifuged for 2 minutes at ~6000 rpm. An aliquot (1.0 mL of dried bean extract, 0.5 mL of orange extract) is made up to a final volume of 10.0 mL with water/acetonitrile (9/1, v/v), mixed and analysed for mandestrobin content by high performance liquid chromatography with tandem mass specific detection (HPLC-MS/MS) in positive electrospray ionisation mode, using a Phenomenex Luna C18 column (50 mm x 2 mm, 5 µm), fitted with a Phenomenex SecurityGuard for C18 HPLC pre-column (2.0 mm), and gradient elution, with a mobile phase of water with 0.1% formic acid and acetonitrile with 0.1% formic acid. Quantification is performed using external standards. The ion transition m/z 314 → m/z 192 is used for quantification and the ion transitions m/z 314 → m/z 160 is used for confirmation.

De-Xy-S-2200: Homogenised samples (20 g) are extracted with acetone/water (4/1, v/v, 50 mL) with mechanical shaking for 10 minutes. The extract is filtered through Celite (40 g) on a Buchner funnel, the original container is rinsed with acetone/water (4/1, v/v, ~15 mL) and the rinsate is also filtered. The residue from the filter is further extracted with acetone/water (4/1, v/v, 50 mL) and the mixture is filtered, container rinsed with acetone/water (4/1, v/v, 15 mL) and the rinsate is also filtered. The filtrates from the two extractions are combined and made to a volume of 200 mL with acetone/water (4/1, v/v). An aliquot (20 mL) is evaporated under vacuum or a stream of air, to a residual aqueous remainder of about 4 mL and is diluted to 20 mL with water. The diluted sample is loaded onto a Chem Elut cartridge and allowed to stand for five minutes. The flask is rinsed four times with dichloromethane (4 x 25 mL) and the rinsates are transferred on to the cartridge. The eluate is collected and evaporated to dryness using a rotary evaporator (40°C). The residues are reconstituted in hexane/acetone (8/1, v/v, 3 mL) using an ultrasonic bath. The extract is cleaned up using a Mega Bond Elut SI cartridge (2 g, 12 mL). The cartridge is conditioned with acetone (5 mL) followed by two portions of hexane (2 x 10 mL). The whole extract is transferred through the cartridge and the extract container is rinsed three times (3 mL, 4 mL and 10 mL) with hexane/acetone (8/1, v/v,) and the rinsates are loaded onto the cartridge. The cartridge is washed with hexane/acetone (2/1, v/v, 10 mL) and

the eluate is discarded. De-Xy-S-2200 is eluted with hexane/acetone (1/2, v/v, 10 mL) into a flask and evaporated to dryness using a rotary evaporator (40°C). The sample is reconstituted with acetonitrile/water (1/2, v/v, 20 mL) using an ultrasonic bath. The reconstituted sample is analysed for content of De-Xy-S-2200 by high performance liquid chromatography with tandem mass specific detection (HPLC-MS/MS) in positive electrospray mode, using a Phenomenex Luna C18 column (150 mm x 2 mm, 5 µm particle size), fitted with a Phenomenex SecurityGuard for C18 HPLC pre-column (2.0 mm), and gradient elution with a mobile phase of 0.1% acetic acid in acetonitrile and 0.1% acetic acid in water. Quantification is performed using external standards monitoring ion transitions m/z 210 \rightarrow m/z 192 and m/z 210 \rightarrow m/z 132 for quantification and confirmation, respectively.

4-OH-S-2200, 2-CH₂OH-S-2200:

Original method: Homogenised samples (20 g) are extracted with acetone/water (4/1, v/v, 50 mL) with mechanical shaking for 10 minutes.

The extract is filtered through Celite (40 g) on a Buchner funnel, the original container is rinsed with acetone/water (4/1, v/v, 25 mL) and the rinsate is also filtered. The residue on the filter is further extracted with acetone/water (4/1, v/v, 50 mL) and the mixture is filtered, rinsed with acetone/water (4/1, v/v, 25 mL) and the rinsate is also filtered. The filtrates from the two extractions are combined and made to a volume of 200 mL with acetone/water (4/1, v/v). An aliquot (20 mL) is evaporated using a rotary evaporator (40°C), to a residual aqueous remainder of 4 mL. The pH of the aqueous remainder is adjusted to 11 with 0.1 M sodium hydroxide and the sample is kept at these basic conditions for 1 hour. The pH of the solution is adjusted to 7 with 0.1 M HCl. Sodium acetate-acetic acid buffer solution (pH5, 10 mM, 10 mL) and β -glucosidase (40 mg) are added, and the sample is shaken for three hours at 100 rpm in a water bath (37°C). The hydrolysed extract is loaded onto a Chem Elut cartridge and allowed to stand for five minutes. The flask is rinsed four times with ethyl acetate (4 x 25 mL) and the rinsates are transferred on to the cartridge. The eluate is collected and evaporated to dryness using a rotary evaporator (40°C). The residues are reconstituted in water (3 mL) using an ultrasonic bath. The extract is cleaned up using an Oasis HLB cartridge (500 mg, 12 mL). The cartridge is conditioned with acetonitrile (5 mL) followed by water (10 mL). The whole extract is transferred through the cartridge and the extract container is rinsed twice (3 mL and 4 mL) with water, and then with 10 mL of water/acetonitrile (4/1, v/v,) using an ultrasonic bath. The rinsates are loaded onto the cartridge. The analytes are eluted with water/acetonitrile (1/1, v/v, 20 mL, 10 mL for day 0 samples) and made up to 20 mL (10 mL for day 0 samples) with water/acetonitrile (1/1, v/v). For day 0 samples, final extracts (10 mL) are further diluted with water/acetonitrile (1/1, v/v). Extracts are analysed for content of 4-OH-S-2200 and 2-CH₂OH-S-2200 by high performance liquid chromatography with tandem mass specific detection (HPLC-MS/MS) in positive and negative electrospray mode, using a Phenomenex Luna C18(2) column (150 mm x 2 mm, 5 µm particle size), fitted with a Phenomenex SecurityGuard for C18 HPLC pre-column (2.0 mm), and gradient elution with a mobile phase of 0.1% acetic acid in acetonitrile and 0.1% acetic acid in water. Quantification was performed using external calibration standards. The 4-OH-S-2200 ion transition m/z 328 \rightarrow m/z 136 (negative ionisation) was used for quantification and the ion transition m/z 330 \rightarrow m/z 192 (positive ionisation) was used for confirmation. The 2-CH₂OH-S-2200 ion transition m/z 330 \rightarrow m/z 192 (positive ionisation) was used for quantification and the ion transition m/z 330 \rightarrow m/z 119 (positive ionisation) was used for confirmation.

Modified method (dried bean samples analysed 3 months onwards): Homogenised samples (20 g) are extracted with sodium ascorbate (1 M, 4 mL), water (12 mL) and acetone (50 mL) on a high-speed mixer for 3 minutes at high speed and then shaken on a reciprocate shaker for 10 minutes.

The extract is filtered through Celite (40 g) on a Buchner funnel, the original container is rinsed with acetone/1 M sodium ascorbate/water (50/4/12, v/v, 15 mL) and the rinsate is also filtered. The residue on the filter is further extracted with acetone/1 M sodium ascorbate/water (50/4/12, v/v, 50 mL) and the mixture is filtered, container rinsed with acetone/1 M sodium ascorbate/water (50/4/12, v/v, 25 mL) and the rinsate is also filtered. The filtrates from the two extractions are combined and made to a volume of 200 mL with acetone/1 M sodium ascorbate/water (50/4/12, v/v). An aliquot (20 mL) is evaporated using a rotary evaporator (40°C), to a residual aqueous remainder of 4 mL. The pH of the aqueous remainder is adjusted to 11 with 0.1 M sodium hydroxide and the sample is kept at these basic conditions for 1 hour. The pH of the

solution is adjusted to 7 with 0.1 M HCl. Sodium acetate-acetic acid buffer solution (pH5, 10 mM, 10 mL) and β -glucosidase (40 mg) are added, and the sample is shaken for three hours at 100 rpm in a water bath (37°C). The hydrolysed extract is loaded onto a Chem Elut cartridge and allowed to stand for 5 minutes. The flask is rinsed four times with ethyl acetate (4 x 25 mL) and the rinsates are transferred on to the cartridge. The eluate is collected and evaporated to dryness using a rotary evaporator (40°C). The residues are reconstituted in water (3 mL) using an ultrasonic bath. The extract is cleaned up using an Oasis HLB cartridge (500 mg, 12 mL). The cartridge is conditioned with acetonitrile (5 mL) followed by water (10 mL). The whole extract is transferred through the cartridge and the extract container is rinsed twice (3 mL and 4 mL) with water, and then with 10 mL of water/acetonitrile (4/1, v/v), using an ultrasonic bath. The rinsates are loaded onto the cartridge. The analytes are eluted with water/acetonitrile (1/1, v/v, 20 mL) and made up to 20 mL (10 mL for day 0 samples) with water/acetonitrile (1/1, v/v). Extracts are analysed for content of 4-OH-S-2200 and 2-CH₂OH-S-2200 by high performance liquid chromatography with tandem mass specific detection (HPLC-MS/MS) in positive and negative electrospray mode, using a Phenomenex Luna C18(2) column (150 mm x 2 mm, 5 μ m particle size), fitted with a Phenomenex SecurityGuard for C18 HPLC pre-column (2.0 mm), and gradient elution with a mobile phase of 0.1% acetic acid in acetonitrile and 0.1% acetic acid in water. Quantification was performed using external calibration standards. The 4-OH-S-2200 ion transition m/z 328 \rightarrow m/z 136 (negative ionisation) was used for quantification and the ion transition m/z 330 \rightarrow m/z 192 (positive ionisation) was used for confirmation. The 2-CH₂OH-S-2200 ion transition m/z 330 \rightarrow m/z 192 (positive ionisation) was used for quantification and the ion transition m/z 330 \rightarrow m/z 119 (positive ionisation) was used for confirmation.

Results and discussions

Table A 20: Recovery results from method validation of mandestrobin, De-Xy-S-2200, 4-OH-S-2200 and 2-CH₂OH-S-2200 using the analytical method

Matrix	Analyte	Ion Transition (m/z)	Fortification Level (mg/kg)	Recoveries (%)	Mean Recovery (%)	RSD (%)
Dried bean (white seeds)	Mandestrobin	314 \rightarrow 192	0.10	87, 94, 96	92	5.1
	De-Xy-S-2200	210 \rightarrow 192	0.10	88, 90, 86	88	2.3
	4-OH-S-2200	328 \rightarrow 136	0.10	76, 64, 73	71	8.8
	2-CH ₂ OH-S-2200	330 \rightarrow 192	0.10	97, 94, 98	96	2.2
Dried bean (white seeds, modified method)	4-OH-S-2200	328 \rightarrow 136	0.01	100, 91, 97	96	4.8
			0.10	81, 79, 83	81	2.5
	2-CH ₂ OH-S-2200	330 \rightarrow 192	0.01	96, 83, 91	90	7.6
			0.10	95, 83, 92	90	6.9
Orange (whole fruit)	Mandestrobin	314 \rightarrow 192	0.10	88, 91, 87	89	2.3
	De-Xy-S-2200	210 \rightarrow 192	0.01	91, 85, 91, 93, 91	90	3.4
			0.10	90, 84, 87, 83, 76	84	6.2
		210 \rightarrow 132	0.01	94, 86, 95, 92, 92	92	3.8
			0.10	92, 85, 88, 83, 75	85	7.5
	4-OH-S-2200	328 \rightarrow 136	0.01	83, 86, 84, 93, 93	88	5.5
			0.10	74, 81, 89, 89, 97	86	10
		330 \rightarrow 192	0.01	87, 86, 83, 91, 102	90	8.2
			0.10	77, 78, 81, 87, 91	83	7.3

Matrix	Analyte	Ion Transition (m/z)	Fortification Level (mg/kg)	Recoveries (%)	Mean Recovery (%)	RSD (%)
	2-CH ₂ OH-S-2200	330 → 192	0.01	99, 98, 103, 101, 106	101	3.2
			0.10	90, 89, 94, 80, 89	88	5.8
		330 → 119	0.01	97, 96, 102, 102, 103	100	3.2
			0.10	91, 89, 94, 92, 101	93	4.9

Table A 21: Recovery results from method validation of mandestrobin, De-Xy-S-2200, 4-OH-S-2200 and 2-CH₂OH-S-2200 using the analytical method

Matrix	Report No.	Analyte	Ion Transition (m/z)	Fortification Level (mg/kg)	Recoveries (%)	Mean Recovery (%)	RSD (%)
Barley straw	ROA-0007	mandestrobin <i>R</i> -isomer (S-2167)	314 → 192	0.005	74, 81, 70, 73, 72	74	5.7
				0.5	71, 80, 79, 88, 76	79	7.9
			314 → 160	0.005	71, 81, 74, 76, 69	74	6.3
				0.5	72, 79, 80, 85, 77	79	6.0
		mandestrobin <i>S</i> -isomer (S-2354)	314 → 192	0.005	87, 92, 87, 91, 81	88	4.9
				0.5	99, 88, 104, 80, 88	92	10
			314 → 160	0.005	84, 84, 83, 96, 80	85	7.2
				0.5	101, 89, 107, 81, 88	93	11
	ROA-0010	De-Xy-S-2200	210 → 132	0.01	88, 85, 85, 88, 88	87	1.9
				0.10	87, 79, 93, 88, 94	88	6.8
			210 → 192	0.01	95, 91, 90, 91, 92	92	2.1
				0.10	87, 81, 93, 89, 95	89	6.2
	ROA-0011	4-OH-S-2200	328 → 136	0.01	100, 82, 112, 100, 110	101	12
				0.10	102, 69, 109, 117, 91	98	19
			330 → 192	0.01	109, 110, 95, 95, 112	104	8.1
				0.10	106, 74, 110, 110, 90	98	16
	ROA-0012	2-CH ₂ OH-S-2200	330 → 192	0.01	78, 99, 86, 80, 76	84	11
				0.10	70, 84, 83, 85, 69	78	10
			328 → 137	0.01	79, 93*, 77, 75, 80	81	8.8
				0.10	77, 80, 76, 84, 80	79	3.9

Table A 22: Characteristics for the analytical method used for validation of mandestrobin, De-Xy-S-2200, 4-OH-S-2200 and 2-CH₂OH-S-2200 residues in dried bean and orange

	Mandestrobin	De-Xy-S-2200	4-OH-S-2200	2-CH ₂ OH-S-2200
Specificity	No residues > 30% of the LOQ were present in untreated samples. Analyte identity was confirmed by comparing the	No residues > 30% of the LOQ were present in untreated samples. Analyte identity was confirmed by comparing the	No residues > 30% of the LOQ were present in untreated samples. Analyte identity was confirmed by comparing the	No residues > 30% of the LOQ were present in untreated samples. Analyte identity was confirmed by comparing the

	Mandestrobin	De-Xy-S-2200	4-OH-S-2200	2-CH₂OH-S-2200
	retention time of the analyte with that of standard reference material.	retention time of the analyte with that of standard reference material.	retention time of the analyte with that of standard reference material.	retention time of the analyte with that of standard reference material.
Calibration (type, number of data points)	Dried bean r=0.9980 Slope=2.74x10 ⁶ Intercept=6.11x10 ⁴ n>7 Orange r=0.9993 Slope=2.01x10 ⁶ Intercept=1.93x10 ⁵ n>7	r=0.9986 Slope=7.19x10 ⁴ Intercept=6.23x10 ³ n>7	r=0.9999 Slope=1.11x10 ⁴ Intercept=-4.90x10 ² n>7	r=0.9986 Slope=5.32x10 ⁵ Intercept=6.11x10 ⁴ n>7
Calibration range	Dried bean Range: 0.075 – 7.5 ng/mL Orange Range: 0.15 – 7.5 ng/mL	Range: 0.25 – 20 ng/mL	Range: 0.3 – 25 ng/mL	Range: 0.3 – 25 ng/mL
Assessment of matrix effects	Matrix effects were found to be insignificant (i.e. <±20%) for dried beans and solvent standards were used. Matrix effects were found to be significant (i.e. >±20%) for orange and matrix-matched standards were used.	Matrix effects were found to be insignificant (i.e. <±20%) and solvent standards were used.	Matrix effects were found to be insignificant (i.e. <±20%) and solvent standards were used.	Matrix effects were found to be insignificant (i.e. <±20%) and solvent standards were used.
Assessment of extract and standard stability	Not assessed			
Limit of detection (LOD)	Dried bean extract: 0.075 ng/mL Orange extract: 0.15 ng/mL	0.25 ng/mL	0.3 ng/mL	0.3 ng/mL
Limit of quantification (LOQ)	0.1 mg/kg	Dried bean: 0.1 mg/kg Orange: 0.01 mg/kg	0.01 mg/kg	0.01 mg/kg

Conclusion

The analytical procedure has been successfully validated in terms of specificity, linearity, accuracy, precision, matrix effects, LOD and LOQ. Although not strictly in accordance with all the requirements of

SANTE/2020/12830, Rev. 2 as extract and standard stability have not been addressed, the acceptable recovery and precision values demonstrate the method is capable of determining mandestrobin, De-Xy-S-2200, 4-OH-S-2200 and 2-CH₂OH-S-2200 in dried beans and orange and the method can be considered fit for purpose.

In study ROR-0286, reduced validation (n=3) is presented at the 0.1 mg/kg level for mandestrobin, De-Xy-S-2200, 4-OH-S-2200 and 2-CH₂OH-S-2200 in dried beans. This LOQ level is appropriate for the storage samples analysed during the pre-registration study and since this method has been previously fully validated on a dry crop matrix at an LOQ of 0.01 mg/kg (see Table A 21), the reduced validation data (n=3) presented at the 0.1 mg/kg level for each analyte in dried beans is considered sufficient to demonstrate the validity of the method for its purpose.

Additionally, reduced validation (n=3) is presented at the 0.1 mg/kg level for mandestrobin in orange. This LOQ level is appropriate for the storage samples analysed during the pre-registration study and since this method has been previously fully validated on a high acid crop matrix at an LOQ of 0.01 mg/kg (see KCP 5.2, Göcer, M., 2012, ROA-0030, EU agreed), the reduced validation data (n=3) presented at the 0.1 mg/kg level in orange is considered sufficient to demonstrate the validity of the method for its purpose.

A 2.1.1.1.11 Analytical method 11

A 2.1.1.1.11.1 Method validation

Comments of zRMS:	The method validation has been accepted. The objective of the study was to retrospectively validate an analytical method, that was previously applied in a storage stability study, for the determination of the mandestrobin metabolites 4-OH-S-2200 and 2-CH ₂ OH-S-2200 in dried beans.
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Reference:	KCP 5.1.2/15 (cross reference to KCA 6.1)
Report	Validation of an Analytical Method for the Determination of the Mandestrobin Metabolites 4-OH-S-2200 and 2-CH ₂ OH-S-2200 in Dried Beans, Linder, M. & Büdel, A., 2020, Report No.: S19-21238 (SUM-1915V), Document No.: ROA-0063
Guideline(s):	SANCO/3029/99, Rev. 4
Deviations:	2 deviations from the study plan with no impact to the study, took place: 1. For the validation of 4-OH-S-2200 and 2-CH ₂ OH-S-2200 in dried beans the lowest concentration chosen for the standard curve was 0.30 ng/mL (0.003 mg/kg) for both analytes which equals 30 % of the LOQ and therefore is above 20 % of the LOQ as requested in the study plan; 2. For the determination of stability of the analytes in the final sample extracts, samples fortified at 10xLOQ level instead of samples fortified at LOQ level, as was requested in the study plan, were used.
GLP:	Yes
Acceptability:	Yes

Materials and methods

Homogenised samples (20 g) are extracted with sodium ascorbate (1 M, 4 mL), water (12 mL) and acetone (50 mL) on a high-speed mixer for 3 minutes at high speed and then shaken on a reciprocate shaker for 10 minutes.

The extract is filtered through Celite (40 g) on a Buchner funnel, the original container is rinsed with acetone/1 M sodium ascorbate/water (50/4/12, v/v, 15 mL) and the rinsate is also filtered. The residue on the

filter is further extracted with acetone/1 M sodium ascorbate/water (50/4/12, v/v, 50 mL) and the mixture is filtered, container rinsed with acetone/1 M sodium ascorbate/water (50/4/12, v/v, 25 mL) and the rinsate is also filtered. The filtrates from the two extractions are combined and made to a volume of 200 mL with acetone/1 M sodium ascorbate/water (50/4/12, v/v). An aliquot (20 mL) is evaporated using a rotary evaporator (40°C), to a residual aqueous remainder of 4 mL. The pH of the aqueous remainder is adjusted to 11 with 0.1 M sodium hydroxide and the sample is kept at these basic conditions for 1 hour. The pH of the solution is adjusted to 7 with 0.1 M HCl. Sodium acetate-acetic acid buffer solution (pH5, 10 mM, 10 mL) and β -glucosidase (40 mg) are added, and the sample is shaken for three hours at 100 rpm in a water bath (37°C). The hydrolysed extract is loaded onto a Chem Elut cartridge and allowed to stand for 3-5 minutes. The flask is rinsed four times with ethyl acetate (4 x 25 mL) and the rinsates are transferred on to the cartridge. The eluate is collected and evaporated to dryness using a rotary evaporator (40°C). The residues are reconstituted in water (3 mL). The extract is cleaned up using an Oasis HLB cartridge (500 mg, 12 mL). The cartridge is conditioned with acetonitrile (5 mL) followed by water (10 mL). The whole extract is transferred through the cartridge and the extract container is rinsed twice (3 mL and 4 mL) with water, and then with 10 mL of water/acetonitrile (4/1, v/v,) using an ultrasonic bath. The rinsates are loaded onto the cartridge. The analytes are eluted with water/acetonitrile (1/1, v/v, 20 mL) and made up to 20 mL with water/acetonitrile (1/1, v/v). Extracts are analysed for content of 4-OH-S-2200 and 2-CH₂OH-S-2200 -by high performance liquid chromatography with tandem mass specific detection (HPLCMS/MS) in positive and negative electrospray mode, using a Phenomenex Luna C18(2) column (150 mm x 2 mm, 5 μ m particle size), fitted with a Phenomenex SecurityGuard for C18 HPLC pre-column (2.0 mm), and gradient elution with a mobile phase of 0.1% acetic acid in acetonitrile and 0.1% acetic acid in water. Quantification was performed using external calibration standards. The 4-OH-S-2200 ion transition m/z 328 \rightarrow 136 (negative ionisation) was used for quantification and the ion transition m/z 330 \rightarrow m/z 192 (positive ionisation) was used for confirmation. The 2-CH₂OH-S-2200 ion transition m/z 330 \rightarrow m/z 192 (positive ionisation) was used for quantification and the ion transition m/z 330 \rightarrow m/z 119 (positive ionisation) was used for confirmation.

Results and discussions

Table A 23: Recovery results from method validation of 4-OH-S-2200 and 2-CH₂OH-S-2200 using the analytical method

Matrix	Analyte	Ion Transition (m/z)	Fortification Level (mg/kg)	Recoveries (%)	Mean Recovery (%)	RSD (%)
Dried beans	4-OH-S-2200	328 \rightarrow 136	0.01	80, 87, 90, 85, 86	86	4.3
			0.10	65, 82, 82, 86, 84	80	11
		330 \rightarrow 192	0.01	86, 85, 95, 92, 90	90	4.6
			0.10	64, 80, 83, 87, 82	79	11
	2-CH ₂ OH-S-2200	330 \rightarrow 192	0.01	72, 60, 84, 79, 80	75	13
			0.10	64, 76, 78, 83, 95	79	14
		330 \rightarrow 119	0.01	86, 77, 96, 92, 85	87	8.3
			0.10	66, 75, 72, 80, 88	76	11

Table A 24: Characteristics for the analytical method used for validation of 4-OH-S-2200 and 2-CH₂OH-S-2200 residues in dried bean

	4-OH-S-2200	2-CH ₂ OH-S-2200
Specificity	No residues > 30% of the LOQ were present in untreated samples. Analyte identity was confirmed by comparing the retention time of the analyte with that of standard reference material. Two ion	No residues > 30% of the LOQ were present in untreated samples. Analyte identity was confirmed by comparing the retention time of the analyte with that of standard reference material. Two ion

	4-OH-S-2200	2-CH₂OH-S-2200
	transitions were monitored by mass spectrometry.	transitions were monitored by mass spectrometry.
Calibration (type, number of data points)	m/z 328 \rightarrow m/z 136 $r=0.9999$ Slope= 2.72×10^4 Intercept=-274 $n=6$ m/z 330 \rightarrow m/z 192 $r=0.9995$ Slope= 2.10×10^5 Intercept=-6618 $n=6$	m/z 330 \rightarrow m/z 192 $r=0.9994$ Slope= 4.39×10^4 Intercept=1214 $n=6$ m/z 330 \rightarrow m/z 119 $r=0.9987$ Slope= 2.91×10^4 Intercept=-1222 $n=6$
Calibration range	Range: 0.30 – 20 µg/L	Range: 0.30 – 20 µg/L
Assessment of matrix effects	Matrix effects were found to be insignificant (i.e. $<\pm 20\%$). Matrix matched calibration standards were used throughout.	Matrix effects were found to be insignificant (i.e. $<\pm 20\%$). Matrix matched calibration standards were used throughout.
Assessment of extract and standard stability	Final extracts of dried beans were found to be stable for 9 days when stored under dark refrigerated (1-10°C) conditions. Stock solutions were found to be stable for 815 days when prepared in acetone and stored under dark refrigerated (1-10°C) conditions. Calibration solutions were found to be stable for 29 days when stored under dark refrigerated (1-10°C) conditions.	Final extracts of dried beans were found to be stable for 9 days when stored under dark refrigerated (1-10°C) conditions. Stock solutions were found to be stable for 815 and 27 days when prepared in acetonitrile and acetone, respectively, and stored under dark refrigerated (1-10°C) conditions. Calibration solutions were found to be stable for 29 days when stored under dark refrigerated (1-10°C) conditions.
Limit of detection (LOD)	0.30 µg/L	0.30 µg/L
Limit of quantification (LOQ)	0.01 mg/kg	0.01 mg/kg

Conclusion

The analytical procedure has been successfully validated in terms of specificity, linearity, accuracy, precision, matrix effects, extract and standard stability, LOD and LOQ. The analytical procedure has been successfully validated in accordance with all of the requirements of SANTE/2020/12830, Rev. 2 for the determination of 4-OH-S-2200 and 2-CH₂OH-S-2200 in dried bean.

A 2.1.1.1.12 Analytical method 12

A 2.1.1.1.12.1 Method validation

Comments of zRMS:	<p>The method validation has been accepted.</p> <p>The analytical method of DX-CA-S-2200 in 0.5% methylcellulose solution was validated to ensure the reliability of analytical method. The calibration curve showed a linear relationship between DX-CA-S-2200 concentration (0.002-0.1 mg/mL) and peak area. Six injections of 0.01 mg/mL standard solution showed an acceptable variability level of injections (the relative standard deviation was 0.457%).</p> <p>In a recovery test, no interfering peaks were observed at the elution position of DX-CA-S-2200 in control samples. Recovery test samples fortified with DX-CA-S-2200 at 1 and 300 mg/mL were analyzed in quintuplicate, and the mean recoveries</p>
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	were 105 and 103% with the relative standard deviations of 0.427 and 0.971%, respectively. The LOQ and LOD were 1 and 0.2 0.002 mg/mL, respectively.
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Reference:	KCP 5.1.2/16 (cross reference to KCA 5.8.1)
Report	Validation of analytical method for DX-CA-S-2200 in 0.5% methylcellulose solution, Shibuya E., 2017, Report No.: 16008VAL, Document No.: ROA-0053
Guideline(s):	SANCO/3029/99, Rev. 4
Deviations:	No assessment of matrix effects or standard stability is presented
GLP:	Yes
Acceptability:	Yes

Materials and methods

Test samples (0.2 mL) are diluted with acetonitrile (to 20 mL) and further into the calibration range with acetonitrile. Samples are analysed by high performance liquid chromatography with ultra-violet detection (HPLC UV) at 254 nm, using a Mightysil RP-18 GP column (150 mm x 4.6 mm, 5 µm particle size) and isocratic elution, with a mobile phase of 0.1% (v/v) trifluoroacetic acid aqueous solution/acetonitrile (80/20, v/v). Quantitation is performed using external standards.

Results and discussions

Table A 25: Recovery results from method validation of DX-CA-S-2200 using the analytical method

Matrix	Analyte	Fortification Level (mg/mL)	Recoveries (%)	Mean Recovery (%)	RSD (%)
0.5% Methyl-cellulose solution	DX-CA-S-2200	1.00	105, 105, 105, 104, 105	105	0.427
		300	102, 102, 104, 104, 103	103	0.971

Table A 26: Characteristics for the analytical method used for validation of DX-CA-S-2200 residues in 0.5% Methylcellulose solution

	DX-CA-S-2200
Specificity	No residues > 30% of the LOQ were present in untreated samples. Analyte identity was confirmed by comparing the retention time of the analyte with that of standard reference material.
Calibration (type, number of data points)	R ² =1.00 Slope=5.93 x 10 ⁵ Intercept=0 n=5
Calibration range	Range: 0.002 – 1.00 mg/mL
Assessment of matrix effects	Not assessed
Assessment of extract and standard stability	Final extracts of 0.5% methylcellulose solution were found to be stable for at least 24 hours at room temperature.
Limit of detection (LOD)	0.002 mg/mL
Limit of quantification (LOQ)	1.00 mg/mL

Conclusion

The analytical procedure has been successfully validated in terms of specificity, linearity, accuracy, precision, extract stability, LOD and LOQ. Although not in accordance with all the requirements of SANTE/2020/12830, Rev. 2, as matrix effects and standard stability have not been assessed, the acceptable recovery and precision values demonstrate this method is capable of determining DX-CA-S-2200 in 0.5% Methylcellulose solution and thus the method can be considered fit for purpose. Additionally, the method satisfies the minimum requirements of an existing method under SANTE/2020/12830, Rev. 2, so can be considered acceptable.

A 2.1.1.1.13 Analytical method 13

A 2.1.1.1.13.1 Method validation

Comments of zRMS:	The method validation has been accepted. This study was conducted with the fungicide S-2200 Technical Grade (active ingredient mandestrobin: 93.1 %). The analytical method was carried out by means of LC-MS/MS in ESI mode to determine of test substance in water.
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Reference:	KCP 5.1.2/17 (cross reference to KCP 10.2.1/02)
Report	Chronic effects of the fungicide Mandestrobin to <i>Daphnia pulex</i> , Roessink, I., 2019a, Report No.: ALT.IR.2018.1, Document No.: ROW-0103
Guideline(s):	SANCO/3029/99, Rev.4.
Deviations:	No assessment of extract and standard stability is presented
GLP:	Yes
Acceptability:	Yes

Materials and methods

Aliquots of freshwater (3 mL) are diluted with acetonitrile (1 mL) and analysed by liquid chromatography with tandem mass specific detection (LC-MS/MS) in positive electrospray ionisation mode using an Agilent analytical Zorbax Eclipse XDB-C18 column (50 mm x 2.1 mm, 3.5 µm particle size) and isocratic elution with a mobile phase of 0.1% formic acid in milli-Q water/0.1% formic acid in acetonitrile (60/40, v/v). Quantification is by external standards monitoring the ion transition m/z 314.18 → 192.1. In addition, confirmatory ion transitions m/z 314.18 → 119 and m/z 314.18 → 132.1 are monitored.

Results and discussions

Table A 27: Recovery results from method validation of mandestrobin using the analytical method

Matrix	Analyte	Ion Transition (m/z)	Fortification Level (mg/L)	Recoveries (%)	Mean Recovery (%)	RSD (%)
Freshwater	Mandestrobin	314.18 → 192.1	0.1	97.5, 97.3, 94.9, 95.5, 97.6, 99.2, 91.9, 95.9, 89.7, 95.6, 98.5, 97.6	95.9	2.87
			0.3	101.1, 97.3, 100.4, 99.3, 97.7, 97.1 105.0, 101.9, 103.2, 104.0	100.7	2.82

Table A 28: Characteristics for the analytical method used for validation of mandestrobin residues in freshwater

	Mandestrobin
Specificity	No residues > 30% of the LOQ were present in untreated samples. Analyte identity was confirmed by comparing the retention time of the analyte with that of standard reference material.
Calibration (type, number of data points)	$R^2=0.9981$ Slope=392220.82 Intercept=0 n=6
Calibration range	Range: 0.8 – 80 ng/mL
Assessment of matrix effects	Matrix effects were found to not be present
Assessment of extract and standard stability	Not assessed
Limit of detection (LOD)	0.8 ng/mL 0.067 ng/mL (the applicant comment: According to report ROW-0103, “The ‘limit of detection’ (LOD; calculated as 3 times the standard deviation of the measured concentrations in the 0.2 ng/mL sample) was 0.067 ng/mL and the LOQ (calculated as 10 times the standard deviation of the measured concentrations in the 0.2 ng/mL sample) was 0.224 ng/ml”). The applicant comment: According to SANTE/2020/12830, Rev. 2, the LOD is defined as the lowest detectable concentration or amount of an analyte in a sample. It should be expressed as lowest calibration standard, therefore the LOD is 0.8 ng/mL.
Limit of quantification (LOQ)	0.1 mg/L 0.224 ng/ml The applicant comment: According to report ROW-0103, “The ‘limit of detection’ (LOD; calculated as 3 times the standard deviation of the measured concentrations in the 0.2 ng/mL sample) was 0.067 ng/mL and the LOQ (calculated as 10 times the standard deviation of the measured concentrations in the 0.2 ng/mL sample) was 0.224 ng/ml”. According to SANTE/2020/12830, Rev. 2, the LOQ is defined as the lowest validated level with sufficient recovery and precision in this case the LOQ is therefore 0.1 mg/L.

Conclusion

The analytical procedure has been successfully validated in terms of specificity, linearity, accuracy, precision, matrix effects, LOD and LOQ. Although not strictly in accordance with all the requirements of SANTE/2020/12830, Rev. 2 as extract and standard stability have not been addressed, the acceptable recovery and precision values demonstrate this method is capable of determining mandestrobin in freshwater and thus the method can be considered fit for purpose. Additionally, the method satisfies the minimum requirements of an existing method under SANTE/2020/12830, Rev. 2, so can be considered acceptable.

A 2.1.1.1.14 Analytical method 14

A 2.1.1.1.14.1 Method validation

Comments of zRMS:	The method validation has been accepted. The purpose of this study was to validate an analytical method used to determine the content of mandestrobin in aqueous solutions. Mandestrobin is composed of two isomers, S-2167 (S-2200 R-isomer) and S-2354 (S-2200 S-isomer), which elute
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	separately under the analytical conditions. Quantitation (LC-MS/MS) was performed separately for the two isomers and the mandestrobin concentration was calculated by the addition of the isomer results. The method was validated to quantify the concentrations of mandestrobin present in recovery samples prepared in moderately hard fortified water. The analytical method was validated with regards to selectivity and specificity, recovery and repeatability, matrix effects, and extract and standard stability.
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Reference:	KCP 5.1.2/18 (cross reference to KCP 10.2.1/03, 10.2.1/07 and 10.2.1/08)
Report	Mandestrobin - Validation of the Analytical Method for the Determination of the Test Substance in Aqueous Solutions, McGuinness, A.M., 2021, Report No.: 13048.7199, Document No.: ROA-0075
Guideline(s):	SANTE/2020/12830, Rev. 1, OCSPP 860.1340
Deviations:	No
GLP:	Yes
Acceptability:	Yes (New data not previously reviewed at EU level, but submitted for mandestrobin renewal of approval (AIR6 dossier) in December 2022)

Materials and methods

Samples are diluted with acetonitrile to a final composition of 50/50 acetonitrile/water (v/v). The 20000 µg/L level samples are further diluted into the calibration range with 50/50 acetonitrile/water (v/v). Samples are analysed for mandestrobin *R*-isomer (S-2167) and mandestrobin *S*-isomer (S-2354) content by high performance liquid chromatography with tandem mass specific detection (HPLC-MS/MS) in positive electrospray ionisation mode, using a Phenomenex Lux Cellulose-3 column (100 mm x 2 mm, 3 µm) and gradient elution, with a mobile phase of water + 0.1% formic acid and acetonitrile + 0.1% formic acid. Quantification is performed using external standards. The ion transition m/z 314.3 > 192.2 is used for quantification and the ion transition m/z 314.3 > 160.0 is used for confirmation.

Results and discussions

Table A 29: Recovery results from method validation of mandestrobin using the analytical method

Matrix	Analyte	Ion Transition (m/z)	Fortification Level (µg/L)	Recoveries (%)	Mean Recovery (%)	RSD (%)
Moderately hard water	Mandestrobin	314.3 > 192.2	0.200	101, 107, 107, 107, 94.0	103	5.54
			20000	106, 109, 109, 108, 108	108	0.69
		314.3 > 160.0	0.200	107, 111, 109, 108, 107	108	1.55
			20000	108, 107, 106, 104, 104	106	1.51

Table A 30: Characteristics for the analytical method used for validation of mandestrobin residues in moderately hard water

	Mandestrobin
Specificity	No residues > 30% of the LOQ were present in untreated samples. Analyte identity was confirmed by comparing the retention time of the analyte with that of standard reference material. Two ion transitions were monitored by mass spectrometry.

	Mandestrobin
Calibration (type, number of data points)	<p>Mandestrobin <i>R</i>-isomer (S-2167) m/z 314.3 \rightarrow 192.2 $r^2=0.998$ Slope=1.18×10^6 Intercept=338 n=6 m/z 314.3 \rightarrow 160.0 $r^2=0.997$ Slope=4.27×10^5 Intercept=1332 n=6</p> <p>Mandestrobin <i>S</i>-isomer (S-2354) m/z 314.3 \rightarrow 192.2 $r^2=0.998$ Slope=1.08×10^6 Intercept=920 n=6 m/z 314.3 \rightarrow 160.0 $r^2=0.996$ Slope=4.55×10^5 Intercept=2968 n=6</p>
Calibration range	Range: 0.01 – 0.1 µg/L
Assessment of matrix effects	Matrix effects were found to be insignificant (i.e. $\leq \pm 20\%$). Matrix matched calibration standards were used throughout.
Assessment of extract and standard stability	Final extracts of moderately hard water were found to be stable for at least 7 days when stored under refrigerated (2-8°C) conditions. Calibration solutions were found to not be stable for 7 days when stored under refrigerated (2-8°C) conditions. Calibration standards should be freshly prepared on the day of use.
Limit of detection (LOD)	0.01 µg/L
Limit of quantification (LOQ)	0.200 µg/L

Conclusion

The analytical procedure has been successfully validated in terms of specificity, linearity, accuracy, precision, matrix effects, extract and standard stability, LOD and LOQ. The analytical procedure has been successfully validated in accordance with all of the requirements of SANTE/2020/12830, Rev. 2 for the determination of mandestrobin in moderately hard water.

A 2.1.1.1.15 Analytical method 15

A 2.1.1.1.15.1 Method validation

Comments of zRMS:	<p>The method has been accepted.</p> <p>This study was conducted with Mandestrobin. The analytical method was carried out by means of LC-MS/MS in ESI mode to determine of test substance in water.</p>
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Reference: KCP 5.1.2/19 (cross reference to KCP 10.2.1/04)

Report	Chronic effects of the fungicide Mandestrobin to <i>Caridina parvidentata</i> , Roessink I., 2019d, Report No.: ALT.IR.2018.7, Document No.: ROW-0106
Guideline(s):	SANCO/3029/99, Rev.4
Deviations:	No assessment of extract and standard stability is presented
GLP:	Yes
Acceptability:	Yes

Materials and methods

Aliquots of freshwater (3 mL) are diluted with acetonitrile (1 mL) and analysed by liquid chromatography with tandem mass specific detection (LC-MS/MS) in positive electrospray ionisation mode using an Agilent analytical Zorbax Eclipse XDB-C18 column (50 mm x 2.1 mm, 3.5 µm particle size) and isocratic elution with a mobile phase of 0.1% formic acid in milli-Q water/0.1% formic acid in acetonitrile (60/40, v/v). Quantification is by external standards monitoring the ion transition m/z 314.18 → 192.1. In addition, confirmatory ion transitions m/z 314.18 → 119 and m/z 314.18 → 132.1 are monitored.

Results and discussions

Table A 31: Recovery results from method validation of mandestrobin using the analytical method

Matrix	Analyte	Ion Transition (m/z)	Fortification Level (mg/L)	Recoveries (%)	Mean Recovery (%)	RSD (%)
Fresh water	Mandestrobin	314.18 → 192.1	0.1	97.0, 95.3, 94.3	95.5	1.43
			0.3	100.3, 101.9, 101.9	101.4	0.91
			1	93.6, 93.8, 92.6	93.3	0.69
			3	94.7, 95.8, 94.4	95.0	0.78
			10	86.1, 84.6, 85.9	85.5	0.95

Table A 32: Characteristics for the analytical method used for validation of mandestrobin residues in fresh water

	Mandestrobin
Specificity	No residues > 30% of the LOQ were present in untreated samples. Analyte identity was confirmed by comparing the retention time of the analyte with that of standard reference material.
Calibration (type, number of data points)	$R^2 = 0.99770751$ Slope=664961.121503 Intercept=0 n=6
Calibration range	Range: 0.8 – 80 ng/mL
Assessment of matrix effects	Matrix effects were found to not be present
Assessment of extract and standard stability	Not assessed
Limit of detection (LOD)	0.8 ng/mL 0.081 ng/mL
Limit of quantification (LOQ)	0.1 mg/L 0.270 ng/mL

	Mandestrobin
	<i>The applicant comment: According to report ROW-0106, “The ‘limit of detection’ (LOD; calculated as 3 times the standard deviation of the measured concentrations in the 0.15 ng/mL sample) was 0.081 ng/mL and the LOQ (calculated as 10 times the standard deviation of the measured concentrations in the 0.15 ng/mL sample) was 0.270 ng/ml”. According to SANTE/2020/12830, Rev. 2, the LOQ is defined as the lowest validated level with sufficient recovery and precision in this case the LOQ is therefore 0.1 mg/L. According to SANTE/2020/12830, Rev. 2, the LOD is defined as the lowest detectable concentration or amount of an analyte in a sample. It should be expressed as lowest calibration standard, therefore the LOD is 0.8 ng/mL.</i>

Conclusion

The analytical procedure has been successfully validated in terms of specificity, linearity, accuracy, precision, matrix effects, LOD and LOQ. Although not strictly in accordance with all the requirements of SANTE/2020/12830, Rev. 2, as extract and standard stability have not been addressed, the acceptable recovery and precision values demonstrate this method is capable of determining mandestrobin in fresh water and thus the method can be considered fit for purpose. Additionally, the method satisfies the minimum requirements of an existing method under SANTE/2020/12830, Rev. 2, so can be considered acceptable.

A 2.1.1.1.16 Analytical method 16

A 2.1.1.1.16.1 Method validation

Comments of zRMS:	The method validation has been accepted. The analytical method was carried out by means of LC-MS/MS to determine concentrations of mandestrobin in water.
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Reference:	KCP 5.1.2/20 (cross reference to KCP 10.2.1/05)
Report	Chronic effects of the fungicide Mandestrobin to <i>Gammarus pulex</i> , Roessink I., 2019c, Report No.: ALT.IR.2018.4, Document No.: ROW-0105
Guideline(s):	SANCO/3029/99, Rev.4
Deviations:	No assessment of extract and standard stability is presented
GLP:	Yes
Acceptability:	Yes

Materials and methods

Aliquots of freshwater (3 mL) are diluted with acetonitrile (1 mL) and analysed by liquid chromatography with tandem mass specific detection (LC-MS/MS) in positive electrospray ionisation mode using an Agilent analytical Zorbax Eclipse XDB-C18 column (50 mm x 2.1 mm, 3.5 µm particle size) and isocratic elution with a mobile phase of 0.1% formic acid in milli-Q water/0.1% formic acid in acetonitrile (60/40, v/v). Quantification is by external standards monitoring the ion transition m/z 314.18 → 192.1. In addition, confirmatory ion transitions m/z 314.18 → 119 and m/z 314.18 → 132.1 are monitored.

Results and discussions

Table A 33: Recovery results from method validation of mandestrobin using the analytical method

Matrix	Analyte	Ion Transition (m/z)	Fortification Level (mg/L)	Recoveries (%)	Mean Recovery (%)	RSD (%)
Fresh water	Mandestrobin	314.18 → 192.1	0.03	97, 92, 107	98.7	7.74
			0.1	95, 98, 95	96.0	1.80
			0.3	101, 109, 94	101.3	7.41
			1	94, 85, 94	91.0	5.71
			3	94, 90, 91	91.7	2.27

Table A 34: Characteristics for the analytical method used for validation of mandestrobin residues in fresh water

	Mandestrobin
Specificity	No residues > 30% of the LOQ were present in untreated samples. Analyte identity was confirmed by comparing the retention time of the analyte with that of standard reference material.
Calibration (type, number of data points)	R ² =0.99734130 Slope=295157.326240 Intercept=0 n=6
Calibration range	Range: 0.8 – 80 ng/mL
Assessment of matrix effects	Matrix effects were found to not be present
Assessment of extract and standard stability	Not assessed
Limit of detection (LOD)	0.8 ng/mL 0.034 ng/mL
Limit of quantification (LOQ)	0.1 mg/L 0.114 ng/ml
	<i>The applicant comment: According to report ROW-0105, “The ‘limit of detection’ (LOD; calculated as 3 times the standard deviation of the measured concentrations in the 0.15 ng/mL sample) was 0.034 ng/mL and the LOQ (calculated as 10 times the standard deviation of the measured concentrations in the 0.15 ng/mL sample) was 0.114 ng/ml”. According to SANTE/2020/12830, Rev. 2, the LOQ is defined as the lowest validated level with sufficient recovery and precision in this case the LOQ is therefore 0.1 mg/L. According to SANTE/2020/12830, Rev. 2, the LOD is defined as the lowest detectable concentration or amount of an analyte in a sample. It should be expressed as lowest calibration standard, therefore the LOD is 0.8 ng/mL.</i>

Conclusion

The analytical procedure has been successfully validated in terms of specificity, linearity, accuracy, precision, matrix effects, LOD and LOQ. Although not strictly in accordance with all the requirements of SANTE/2020/12830, Rev. 2, as extract and standard stability have not been addressed, the acceptable recovery and precision values demonstrate this method is capable of determining mandestrobin in fresh water and thus the method can be considered fit for purpose. Additionally, the method satisfies the minimum requirements of an existing method under SANTE/2020/12830, Rev. 2, so can be considered acceptable.

A 2.1.1.1.17 Analytical method 17

A 2.1.1.1.17.1 Method validation

Comments of zRMS:	The method validation has been accepted. The analytical method was carried out by means of LC-MS/MS to determine concentrations of mandestrobin in water.
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Reference:	KCP 5.1.2/21 (cross reference to KCP 10.2.1/06)
Report	Chronic effects of the fungicide Mandestrobin to <i>Asellus aquaticus</i> , Roessink I., 2019b, Report No.: ALT.IR.2018.2, Document No.: ROW-0104
Guideline(s):	SANCO/3029/99, Rev. 4
Deviations:	No assessment of extract and standard stability is presented
GLP:	Yes
Acceptability:	Yes

Materials and methods

Aliquots of freshwater (3 mL) are diluted with acetonitrile (1 mL) and analysed by liquid chromatography with tandem mass specific detection (LC-MS/MS) in positive electrospray ionisation mode using an Agilent analytical Zorbax Eclipse XDB-C18 column (50 mm x 2.1 mm, 3.5 µm particle size) and isocratic elution with a mobile phase of 0.1% formic acid in milli-Q water/0.1% formic acid in acetonitrile (60/40, v/v). Quantification is by external standards monitoring the ion transition m/z 314.18 → 192.1. In addition, confirmatory ion transitions m/z 314.18 → 119 and m/z 314.18 → 132.1 are monitored.

Results and discussions

Table A 35: Recovery results from method validation of mandestrobin using the analytical method

Matrix	Analyte	Ion Transition (m/z)	Fortification Level (mg/L)	Recoveries (%)	Mean Recovery (%)	RSD (%)
Fresh water	Mandestrobin	314.18 → 192.1	0.03	118, 99, 93	103.3	12.63
			0.1	97, 99, 102	99.3	2.53
			0.3	121, 97, 86	101.3	17.66
			1	87, 112, 93	97.3	13.41
			3	92, 89, 89	90.0	1.92

Table A 36: Characteristics for the analytical method used for validation of mandestrobin residues in fresh water

	Mandestrobin
Specificity	No residues > 30% of the LOQ were present in untreated samples. Analyte identity was confirmed by comparing the retention time of the analyte with that of standard reference material.

	Mandestrobin
Calibration (type, number of data points)	$R^2=0.99901617$ Slope=334755.8888946 Intercept=0 n=6
Calibration range	Range: 0.8 – 80 ng/mL
Assessment of matrix effects	Matrix effects were found to not be present
Assessment of extract and standard stability	Not assessed
Limit of detection (LOD)	0.8 ng/mL 0.034 ng/mL
Limit of quantification (LOQ)	0.03 mg/L 0.114 ng/mL
	The applicant comment: According to report ROW-0104, “The ‘limit of detection’ (LOD; calculated as 3 times the standard deviation of the measured concentrations in the 0.15 ng/mL sample) was 0.034 ng/mL and the LOQ (calculated as 10 times the standard deviation of the measured concentrations in the 0.15 ng/mL sample) was 0.114 ng/mL”. According to SANTE/2020/12830, Rev. 2, the LOQ is defined as the lowest validated level with sufficient recovery and precision in this case the LOQ is therefore 0.1 mg/L. According to SANTE/2020/12830, Rev. 2, the LOD is defined as the lowest detectable concentration or amount of an analyte in a sample. It should be expressed as lowest calibration standard, therefore the LOD is 0.8 ng/mL.

Conclusion

The analytical procedure has been successfully validated in terms of specificity, linearity, accuracy, precision, matrix effects, LOD and LOQ. Although not strictly in accordance with all the requirements of SANTE/2020/12830, Rev. 2, as extract and standard stability have not been addressed, the acceptable recovery and precision values demonstrate this method is capable of determining mandestrobin in fresh water and thus the method can be considered fit for purpose. Additionally, the method satisfies the minimum requirements of an existing method under SANTE/2020/12830, Rev. 2, so can be considered acceptable.

A 2.1.1.1.18 Analytical method 18

A 2.1.1.1.18.1 Method validation

Comments of zRMS:	Accepted
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Reference:	KCP 5.1.2/22 (cross reference to KCP 2.8.3.1 and KCP 2.8.3.2)
Report	Physical and chemical properties and storage stability tests for Mandestrobin 40 SC Third Interim Report, 2025, Lecocq, V., 2025, Report No.: 25610, Document No.: ROF-0025
Guideline(s):	SANTE/2020/12830, Rev.1
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

Supplementary validation of Method 25587 (See KCP 5.1.1/01) is provided below to extend the use of the method for the determination of suspensibility and spontaneity of dispersion. The sample preparation procedure differs as follows:

Suspensibility

Treatment of the remaining tenth for lowest concentration

After removal of the top 225 mL of suspension, transfer the 25 mL remaining in the cylinder into a conical flask. Evaporate to dryness using a rotate evaporator. Dissolve the dry residue with 15 mL of acetonitrile, at $20^{\circ}\text{C} \pm 1^{\circ}\text{C}$. Mix thoroughly, filter through a $0.2\ \mu\text{m}$ (or $0.45\ \mu\text{m}$ depending on the HPLC system) filter and inject in HPLC.

Treatment of the remaining tenth for highest concentration

Add 50 mL of acetonitrile, at $20^{\circ}\text{C} \pm 1^{\circ}\text{C}$, in the 250 mL measuring cylinder containing the remaining tenth. Place the cylinder in an ultrasonic bath for 10 minutes. Mix thoroughly, filter through a $0.2\ \mu\text{m}$ (or $0.45\ \mu\text{m}$) filter and inject in HPLC.

Spontaneity of dispersion

Add 75 mL of acetonitrile, at $20^{\circ}\text{C} \pm 1^{\circ}\text{C}$, in the 250 mL measuring cylinder containing the remaining tenth. Place the cylinder in an ultrasonic bath for 10 minutes. Mix thoroughly. Transfer by pipette 7.5 mL of this solution, at $20^{\circ}\text{C} \pm 1^{\circ}\text{C}$, into a 50 mL volumetric flask and dilute to the mark at $20^{\circ}\text{C} \pm 1^{\circ}\text{C}$ with acetonitrile. Mix well, filter through a $0.2\ \mu\text{m}$ (or $0.45\ \mu\text{m}$ depending on the HPLC system) filter and inject in HPLC.

Sample analysis

Samples are analysed by HPLC-UV. The chromatographic conditions used are an Agilent Zorbax Eclipse XDB-C18 column (150 mm x 4.6 mm, $5\ \mu\text{m}$), isocratic elution with a mobile phase of acetonitrile/water/phosphoric acid (500/500/1, v/v/v) and detection at 275 nm. Quantification is achieved by use of external standards.

Results and discussions

Table A 37: Recovery results from method validation of mandestrobin using the analytical method

Matrix	Analyte	Test	Fortification Level	Recoveries (%)	Mean Recovery (%)	RSD (%)
CIPAC water D	Mandestrobin	Suspensibility	0.1% v/v *	95.2, 95.6, 95.3, 95.2, 96.4	95.5	0.52
			0.5% v/v **	96.3, 96.2, 96.2, 96.3, 96.1	96.2	0.10
		Spontaneity of dispersion	Indice 100%	95.5, 96.1, 95.6, 95.0, 95.9	95.6	0.42
			Indice 60%	95.1, 95.6, 95.3, 95.3, 95.3	95.3	0.21

* Indice of 100%, ** Indice of 60%

Table A 38: Characteristics for the analytical method used for validation of mandestrobin in CIPAC water D for the determination of suspensibility & spontaneity of dispersion

	Mandestrobin
Specificity	No residues > 30% of the LOQ were present in untreated samples. Analyte identity was confirmed by comparing the retention time of the analyte with that of standard reference material.

	Mandestrobin
Calibration (type, number of data points)	$r^2 = 0.9999$ Slope=3602 Intercept=21284 n=5
Calibration range	Range: 473 – 1409 ng/mL
Assessment of matrix effects	Not required
Assessment of extract and standard stability	Not required
Limit of detection (LOD)	473 ng/mL
Limit of quantification (LOQ)	0.1% v/v

Conclusion

The analytical procedure has been successfully validated in terms of specificity, linearity, accuracy, precision, LOD and LOQ. The analytical procedure has been successfully validated in accordance with all of the requirements of SANTE/2020/12830, Rev.2 for the determination of mandestrobin in CIPAC water D.

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

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

A 2.1.2.1.1 Extraction efficiency

Comments of zRMS:	<p>The study has been accepted.</p> <p>The objective of this cross-validation study was to compare residue amounts of mandestrobin (S-2200) extracted from a sample of peach with incurred residues taken from another study (GLP study 491-2021) by extraction as used in the multi-residue monitoring method QuEChERS and by another extraction as was used in a metabolism study. The study was conducted in accordance with SANTE 2017/10632, rev. 4.</p> <p>LC-MS/MS determination was conducted by monitoring two mass transitions. Due to enhanced sensitivity mass transition m/z 314→192 was used for quantification but both mass transitions (m/z 314→192 and m/z 314→160) are applicable interchangeably for quantification and confirmation.</p> <p>With regard to selectivity, accuracy and precision, the analytical procedures were applied successfully for each analytical set when analysing the incurred residue sample of GLP Study 491-2021.</p> <p>Since the residue amounts of mandestrobin in peach obtained with the QuEChERS differed by no more than 30 % compared to the results obtained with the solvents of the metabolism study the extraction efficiency of the QuEChERS based extraction can be considered to be proven.</p> <p>The references used: [2] EN 15662:2018: "Foods of plant origin – Multimethod for the determination of pesticide residues using GC- and LC-based analysis following acetonitrile extraction/partitioning and clean-up by dispersive SPE – Modular QuEChERS-method ". [3] Study No. 13048.6631: "Metabolism of [^{14}C]S-2200 in Lettuce Plants", USA (2010).</p>
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Reference:	KCP 5.2/01
Report	Cross-Validation - Comparing Amounts of Mandestrobin extracted from Samples of Peach with incurred Residues using two different Solvent Systems, Lindner, M. & Fiedler, S., 2022, Document No.: S21-00874 (SUM-2101V), Report No.: ROA-0085
Guideline(s):	SANTE/2017/10632, rev. 4 SANTE/2020/12830, rev. 1
Deviations:	No negative impact: Due to a mistake retain sample “491-2021 SP01 2R” was analysed instead of “491-2021 SP01 2” as was foreseen in the Study Plan.
GLP:	Yes
Acceptability:	Yes (New data not previously reviewed at EU level, but submitted for mandestrobin renewal of approval (AIR6 dossier) in December 2022)

Residue Method

Materials and methods

Samples (10 g) are shaken vigorously for 1 minute with acetonitrile (10 mL) in a 50 mL centrifuge vial. Magnesium sulfate (4 g), sodium chloride (1 g), trisodium citrate dihydrate (1 g) and disodium hydrogencitrate sesquihydrate (0.5 g) are added (in the form of a premixed dispersive SPE tube) and the contents are shaken for 1 minute and centrifuged for 5 minutes at ~3200 x g. PSA (37.5 mg) and magnesium sulfate (225 mg) are weighed into a safe-lock tube, to which an aliquot (~1.5 mL) of the supernatant is added. The tube is shaken vigorously by hand for 30 seconds and centrifuged for 5 minutes at ~3500 x g. An aliquot (0.50 mL) is made up to a final volume of 10.0 mL with water/acetonitrile (9/1, v/v), vortex mixed and analysed for mandestrobin content by high performance liquid chromatography with tandem mass specific detection (HPLC-MS/MS) in positive electrospray ionisation mode, using an Agilent Luna C18(2) column (50 mm x 2 mm, 5 µm) and gradient elution, with a mobile phase of water + 0.1% formic acid and acetonitrile + 0.1% formic acid. Quantification is performed using external standards. The ion transition m/z 314 > 192 is used for quantification and the ion transitions m/z 314 > 160 is used for confirmation.

Results and discussions

Table A 39: Recovery results from method validation of mandestrobin using the analytical method

Matrix	Analyte	Ion Transition (m/z)	Fortification Level (mg/kg)	Recoveries (%)	Mean Recovery (%)	RSD (%)
Peach (fruit without stone)	Mandestrobin	314 > 192	0.01	107, 112, 106, 115, 105	109	4.0
			0.1	111, 107, 107, 104, 108	107	2.4
			0.5	107, 108, 109	108	0.9
		314 > 160	0.01	107, 109, 108, 113, 107	109	2.1
			0.1	109, 104, 106, 106, 106	106	1.7
			0.5	109, 108, 108	108	0.5

Table A 40: **Characteristics for the analytical method used for validation of mandestrobin residues in peach**

	Mandestrobin
Specificity	HPLC-MS/MS monitoring two ion transitions is a highly specific technique. Analysis of control matrix demonstrated that no significant interferences (>30% of the LOQ) were present at the retention time of mandestrobin. Analyte identity was confirmed by retention time match with the analytical standard.
Calibration (type, number of data points)	<p>314 > 192 r = 0.9995 Slope = 3.03×10^5 Intercept = 7745 n = 8</p> <p>314 > 160 r = 0.9999 Slope = 1.46×10^5 Intercept = 3079 n = 8</p>
Calibration range	0.15 – 10 ng/mL
Assessment of matrix effects is presented	Matrix effects were found to be insignificant. Matrix-matched standards were used for quantification.
Assessment of extract and standard stability	Final extracts were found to be stable for at least 14 days when stored under dark refrigerated (1-10°C) conditions. Calibration standards were analysed within 24 hours of preparation. Stock solutions were found to be stable for at least 245 days stored under dark refrigerated (1-10°C) conditions.
Limit of detection (LOD)	0.15 ng/mL
Limit of quantification (LOQ)	0.01 mg/kg

Metabolism Method

Materials and methods

Acetonitrile (40-45 mL) is added to the homogenised sample (5.0 g ± 0.1 g), the sample shaken on a high-speed homogeniser for 1 minute and shaken on a horizontal shaker for 10 minutes at ~200 rot/min. The sample is then centrifuged for ~5 minutes at ~3200 x g. The supernatant is decanted. Acetone/water (4/1, v/v, 40-45 mL) is added to the remaining material in the centrifuge tube, this is shaken on a horizontal shaker for 10 minutes at ~200 rot/min. The sample is then centrifuged for ~5 minutes at ~3200 x g. The supernatant is decanted to join the first supernatant. Acetone/water (4/1, v/v, 40-45 mL) are added to the remaining material in the centrifuge tube, this is shaken on a horizontal shaker for 10 minutes at ~200 rot/min. The sample is then centrifuged for ~5 minutes at ~3200 x g. The supernatant is decanted to join the first and second supernatant. The combined extracts are diluted to 150 mL with acetone/water (4/1, v/v) and briefly mixed with a glass rod. An aliquot (50 mL) and is evaporated to its aqueous phase (~6 mL) under a stream of nitrogen and a water bath set at ~40°C. Acetonitrile (10 mL) is added to the aqueous remainder and the sample is shaken vigorously by hand for a minimum of 1 minute followed by shaking on a platform shaker for 15 minutes. Magnesium sulfate (4.0 g), sodium chloride (1.0 g), trisodium citrate dihydrate (1.0 g) and disodium hydrogen citrate sesquihydrate (0.5 g) are added. The sample is immediately shaken by hand for 1 minute and centrifuged for ~5 minutes at ~3200 x g. PSA (37.5 mg) and magnesium sulfate (225 mg) are weighed into a 2 mL tube, to which an aliquot (~1.5 mL) of the supernatant is added. The tube is shaken vigorously by hand for 30 seconds and centrifuged for 5

minutes at ~3500 x g. An aliquot (0.75 mL) is made up to a final volume of 2.5 mL with water and analysed for mandestrobin content by high performance liquid chromatography with tandem mass specific detection (HPLC-MS/MS) in positive electrospray ionisation mode, using an Agilent Luna C18(2) column (50 mm x 2 mm, 5 µm) and gradient elution, with a mobile phase of water + 0.1% formic acid and acetonitrile + 0.1% formic acid. Quantification is performed using external standards. The ion transition m/z 314 > 192 is used for quantification and the ion transitions m/z 314 > 160 is used for confirmation.

Results and discussions

Table A 41: Recovery results from method validation of mandestrobin using the analytical method

Matrix	Analyte	Ion Transition (m/z)	Fortification Level (mg/kg)	Recoveries (%)	Mean Recovery (%)	RSD (%)
Peach (fruit without stone)	Mandestrobin	314 > 192	0.01	85, 92, 82, 91, 90	88	4.9
			0.1	95, 98, 93, 89, 90	93	4.0
			0.5	99, 87, 97	94	7.0
		314 > 160	0.01	90, 92, 89, 84, 91	89	3.6
			0.1	95, 93, 91, 92, 88	92	2.8
			0.5	97, 89, 93	93	4.3

Table A 42: Characteristics for the analytical method used for validation of mandestrobin residues in peach

	Mandestrobin
Specificity	HPLC-MS/MS monitoring two ion transitions is a highly specific technique. Analysis of control matrix demonstrated that no significant interferences (>30% of the LOQ) were present at the retention time of mandestrobin. Analyte identity was confirmed by retention time match with the analytical standard.
Calibration (type, number of data points)	314 > 192 $r = 0.9994$ $\text{Slope} = 1.85 \times 10^5$ $\text{Intercept} = 2437$ $n = 8$ 314 > 160 $r = 0.9993$ $\text{Slope} = 9.42 \times 10^4$ $\text{Intercept} = 1739$ $n = 8$
Calibration range	0.15 – 10 ng/mL
Assessment of matrix effects is presented	Matrix effects were found to be insignificant. Matrix-matched standards were used for quantification.
Assessment of extract and standard stability	Final extracts were found to be stable for at least 8 days when stored under dark refrigerated (1-10°C) conditions.

	Mandestrobin
Limit of detection (LOD)	0.15 ng/mL
Limit of quantification (LOQ)	0.01 mg/kg

Extraction Efficiency

The individual extraction efficiencies (relative recovery) of the metabolism and residue method extractions are given in the table below.

Table A 43: Extraction Efficiency Data

Matrix	Analyte	Method	Residue Extracted (mg/kg)	Relative Recovery (%)	Difference (%)
Peach	Mandestrobin	Residue	0.30	111	11
		Metabolism	0.27	100	

The residue and metabolism extraction procedure showed equivalent (difference $\leq 30\%$) extraction efficiency for mandestrobin in peach.

Conclusion

According to SANTE/2017/10632, rev. 4 the extraction efficiency of the QuEChERS based extraction procedure can formally be considered as being sufficiently proven because the residue amounts of mandestrobin in the incurred residue sample of peach obtained with the QuEChERS-based approach differed by no more than 30% compared to the results obtained with the solvents of the metabolism study.

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

No new or additional studies have been submitted.

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

No new or additional studies have been submitted.

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

A 2.1.2.4.1.1 Independent laboratory validation

Comments of zRMS:	The independent method validation has been accepted.
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Reference:

KCA 5.2/02

Report

Independent Laboratory Validation of an Analytical Method for Determination of Mandestrobin in Drinking Water, Lindner, M. & Grewe, D., 2021, Report No.: S21-00875 (SUM-2102V), Document No.: ROA-0068

Guideline(s):

SANTE/2020/12830 Rev.1.

Deviations:

No

GLP: Yes

Acceptability: Yes

Materials and methods

An aliquot (1.0 mL) of drinking water is mixed with acetonitrile (100 µL) by vortex and analysed for mandestrobin content by high performance liquid chromatography with tandem mass specific detection (HPLC-MS/MS) in positive electrospray ionisation mode, using a Phenomenex C18 Luna column (50 mm x 2.0 mm, 5 µm particle size) and gradient elution with a mobile phase of 0.1% formic acid in acetonitrile and 0.1% formic acid in water. Quantification is performed using external standards monitoring ion transitions m/z 314 > 192 and m/z 314 > 160 for quantification and confirmation, respectively.

Results and discussions

Table A 44: Recovery results from method validation of mandestrobin using the analytical method

Matrix	Analyte	Ion Transition (m/z)	Fortification Level (µg/L)	Recoveries (%)	Mean Recovery (%)	RSD (%)
Drinking water	mandestrobin	314 > 192	0.1	107, 109, 109, 104, 114	109	3.3
			1.0	106, 108, 102, 105, 109	106	2.6
		314 > 160	0.1	105, 109, 109, 107, 112	109	2.4
			1.0	105, 109, 105, 105, 109	107	2.1

Table A 395: Characteristics for the analytical method used for validation of mandestrobin residues in drinking water

	Mandestrobin
Specificity	No residues > 30% of the LOQ were present in untreated samples. Analyte identity was confirmed by comparing the retention time of the analyte with that of standard reference material. Two ion transitions were monitored by mass spectrometry.
Calibration (type, number of data points)	m/z 314 → 192 $r=0.9990$ Slope= 6.20×10^5 Intercept=1103 $n=8$ m/z 314 → 160 $r=0.9993$ Slope= 1.61×10^5 Intercept=137.1 $n=8$
Calibration range	Range: 0.0175 – 2.0 ng/mL
Assessment of matrix effects	Matrix effects were found to be insignificant (i.e. <±20%). Matrix matched calibration standards were used throughout.
Assessment of extract and standard stability	Since no extraction is performed on drinking water samples, storage stability of extracts is not applicable. Stock and calibration solutions were found to be stable for at least 1583 days and 11 days, respectively, when stored under dark refrigerated (1-10°C) conditions.
Extraction efficiency	Not required.

	Mandestrobin
Limit of detection (LOD)	0.0175 ng/mL
Limit of quantification (LOQ)	0.1 µg/L

Conclusion

The analytical procedure has been successfully validated in terms of specificity, linearity, accuracy, precision, matrix effects, extract and standard stability, LOD and LOQ. The analytical procedure has been successfully validated as an ILV in accordance with all of the requirements of SANTE/2020/12830, Rev.2 for the determination of mandestrobin in drinking water.

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:	The method validation has been accepted.
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Reference: KCA 5.2/05

Report Validation of an Analytical Method for the Determination of 5-COOH-S-2200 in Blood Plasma and Liver (with amendment No. 1), Lindner, M., 2021, Report No.: S21-00876 (SUM-2103V), Document No.: ROA-0072

Guideline(s): SANTE/2020/12830 Rev.1.

Deviations: No

GLP: Yes

Acceptability: Yes

Materials and methods

Bovine liver: Water (6.0 mL) and 1% formic acid in acetonitrile (10 mL) are added to homogenised liver samples (5.0 ± 0.05 g). The sample is shaken vigorously by hand for 1 minute and for a further 15 minutes on a platform shaker. Magnesium sulfate (4.0 g) and sodium chloride (1.0 g) are added, the sample is shaken vigorously by hand for 1 minute and centrifuged for 5 minutes at $\sim 3200 \times g$. An aliquot (~ 8.0 mL) of the upper acetonitrile phase is isolated and frozen for at least 3 hours at $\leq -18^\circ\text{C}$. The cooled extracts are centrifuged for ~ 1 minute at $\sim 3200 \times g$. C18(ec) (37.5 mg) and magnesium sulfate (225 mg) are weighed into a safe-lock tube, to which an aliquot (~ 1.5 mL) of the supernatant is added. The tube is shaken vigorously by hand, by vortex for 30 seconds and centrifuged for 5 minutes at $\sim 3200 \times g$. An aliquot (500 µL) is made up to a final volume of 5.0 mL with water.

Bovine blood plasma: Water (1.5 mL) and acetonitrile (2 mL) are added to blood plasma samples (0.5 mL). The sample is shaken vigorously by hand for 1 minute and for a further 15 minutes on a platform shaker at 250 rpm. Magnesium sulfate (0.8 g), sodium chloride (0.2 g), trisodium citrate dihydrate (0.2 g) and disodium hydrogen sesquihydrate (0.1 g) are added, the sample is shaken vigorously by hand for 1 minute and centrifuged for 5 minutes at ~3200 x g. An aliquot (1.0 mL) is made up to a final volume of 5.0 mL with water.

Extracts are analysed for 5-COOH-S-2200 content by high performance liquid chromatography with tandem mass specific detection (HPLC-MS/MS) in positive electrospray ionisation mode, using an ACE 3 C18-PFP column (150 mm x 2.1 mm, 3 µm particle size) and gradient elution with a mobile phase of 0.1% formic acid in acetonitrile and 0.1% formic acid in water. Quantification is performed using external standards monitoring ion transitions m/z 344 > 192 and m/z 344 > 160 for quantification and confirmation, respectively.

Results and discussions

Table A 406: Recovery results from method validation of 5-COOH-S-2200 using the analytical method

Matrix	Analyte	Ion Tran- sition (m/z)	Fortification Level	Recoveries (%)	Mean Re- covery (%)	RSD (%)
Bovine liver	5-COOH-S- 2200	344 > 192	0.01 mg/kg	80, 78, 75, 80, 78	78	2.7
			0.1 mg/kg	80, 79, 83, 80, 78	80	2.1
		344 > 160	0.01 mg/kg	81, 77, 74, 78, 75	77	3.6
			0.1 mg/kg	83, 82, 86, 82, 81	83	2.5
Bovine blood plasma		344 > 192	0.01 mg/L	71, 79, 73, 85, 83	78	7.5
			0.1 mg/L	93, 101, 91, 110, 104	100	7.8
		344 > 160	0.01 mg/L	70, 79, 75, 84, 83	78	7.5
			0.1 mg/L	92, 99, 93, 110, 105	100	7.7

Table A 417: Characteristics for the analytical method used for validation of 5-COOH-S-2200 residues in bovine liver and blood plasma

	5-COOH-S-2200	
	Bovine liver	Bovine blood plasma
Specificity	No residues > 30% of the LOQ were present in untreated samples. Analyte identity was confirmed by comparing the retention time of the analyte with that of standard reference material. Two ion transitions were monitored by mass spectrometry.	No residues > 30% of the LOQ were present in untreated samples. Analyte identity was confirmed by comparing the retention time of the analyte with that of standard reference material. Two ion transitions were monitored by mass spectrometry.
Calibration (type, number of data points)	m/z 344 → 192 r=0.9999 Slope=4.78 x 10 ⁵ Intercept=4632 n=6 m/z 344 → 160 r=0.9998 Slope=2.17 x 10 ⁵ Intercept=4935	m/z 344 → 192 r=0.9999 Slope=3.28 x 10 ⁵ Intercept=-6687 n=6 m/z 344 → 160 r=0.9997 Slope=1.55 x 10 ⁵ Intercept=-3689

	5-COOH-S-2200	
	Bovine liver	Bovine blood plasma
	n=6	n=6
Calibration range	Range: 0.1 – 10 ng/mL	Range: 0.1 – 10 ng/mL
Assessment of matrix effects	Matrix effects were found to be significant (i.e. >±20%). Matrix matched calibration standards were used throughout.	Matrix effects were found to be insignificant (i.e. <±20%). Matrix matched calibration standards were used throughout.
Assessment of extract and standard stability	Extracts were found to be stable for 14 days in final extracts of bovine liver, when stored under dark refrigerated (1-10°C) conditions. Stock and calibration solutions were found to be stable for at least 18 days and 11 days, respectively, when stored under dark refrigerated (1-10°C) conditions.	Extracts were found to be stable for 7 days in final extracts of bovine blood plasma, when stored under dark refrigerated (1-10°C) conditions. Stock and calibration solutions were found to be stable for at least 18 days and 11 days, respectively, when stored under dark refrigerated (1-10°C) conditions.
Limit of detection (LOD)	0.1 ng/mL	0.1 ng/mL
Limit of quantification (LOQ)	0.01 mg/kg	0.01 mg/L

Conclusion

The analytical procedure has been successfully validated in terms of specificity, linearity, accuracy, precision, matrix effects, extract and standard stability, LOD and LOQ. The analytical procedure has been successfully validated in accordance with all of the requirements of SANTE/2020/12830, Rev.2 for the determination of 5-COOH-S-2200 in bovine liver and blood plasma.

A 2.1.2.7 Other Studies/ Information

No new or additional studies have been submitted.