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24-EPIBRASSINOLIDE

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B.5. METHODS OF ANALYSIS

B.5.1. METHODS USED FOR THE GENERATION OF PRE-AUTHORISATION DATA (CA 4.1)

B.5.1.1. Methods for the analysis of the active substance as manufactured (CA 4.1.1)

Reference:	**PRELIMINARY ANALYSIS AND ENFORCEMENT ANALYTICAL METHOD OF 24-EPIBRASSINOLIDE TGAI**
Author(s), year:	Gao J. (2017)
Report/Doc. number:	NC-2015-032 (172-001)
Guideline(s):	SANCO/3030/99-rev.4
GLP:	Yes

Different methods are included in this study for the determination of the active substance and the impurities. Only the method for 24-Epibrassinolide is discussed here, the other methods (for the impurities) are discussed in Volume 4.

Material and methods:

Test material:
Technical batch of 24-Epibrassinolide

Analyte:
24-Epibrassinolide

Principle of the method:
Active substance content is determined after dissolving the test item in Methanol and derivatization by adding Phenyboronic acid. The separation is achieved using HPLC with UV detection (at 220 nm).

HPLC Conditions: Agilent ZORBAX XDB-C8 column, 5 µm particle size; Acetonitrile /water (78 : 22 v/v); static mode

Findings:

- Specificity: The specificity of the method was demonstrated by comparison of the retention time with the reference item and by analysis of a reagent blank. No interferences were detected.
- Calibration (Linearity): The linearity was determined from 6 levels of standard covering the validation range relevant to the concentration of the active substance. The calibration curve was linear within the measured range of 197 to 599 mg/L, equivalent to a 24-Epibrassinolide concentration of 49 to 150% (w/w).
- Accuracy (Recovery): This method was considered to have satisfactory accuracy from the results of specificity, linearity and precision.
- Precision (Repeatability): The relative standard deviation was calculated from 6 seperate determinations of the content of 24-Epibrassinolide technical. The method is suitably precise with mean relative standard deviations inside the recommended values (modified Horwitz value RSDr). No outlier was discarded.
- Confirmation of identification: Analyte confirmation was shown via UV chromatograms, TIC, UV spectrum, NMR, IR and MS spectrum of the test substance compared to analytical reference standard.

Conclusion:
The method is acceptable and allows the determination of the active substance 24-Epibrassinolide in the technical material.

Table 5.1.1-1: Validation data summary for the determination of 24-Epibrassinolide in the technical material

References	Method	Analyte	Specificity / interferences	Calibration	Precision
Gao J.	HPLC-UV	24-Epibrassinolide	No interferences	n = 6 49 – 150 %w/w R > 0.999	n = 6 RSD 0.63 % RSD_r 1.36 %

Applicability of existing CIPAC methods

No CIPAC method is available for the determination of 24-Epibrassinolide in technical material.

B.5.1.2. Methods for risk assessment (CA 4.1.2)

The references of all risk assessment methods are located in the respective sections. Please note that the reliabilities of the corresponding methods are considered in the relevant sections of the risk assessment, if necessary.

B.5.1.2.1. *Physical and chemical properties Data*

Following (non-radiolabelled) analytical methods used in risk assessment studies were evaluated in this chapter.

Reference:	**KCA 4.1.2/01, CHEMICAL AND PHYSICAL CHARACTERIZATION OF 24-EPIBRASSINOLIDE TGAI**
Author(s), year:	Gao J. (2016)
Report/Doc. number:	NC-2015-033 (119-001)
Guideline(s):	-
GLP:	yes

Test item

aqueous solution

Analyte

24-Epibrassinolide as derivate (derivatised with phenylboronic acid)

Principle of method

HPLC-UV

Validation

Specificity/Interference

The specificity of the analytical method was verified by checking the retention time of the analyte. Control value is < 30% LOQ

Linearity

A calibration curve is constructed by injecting standard solutions within the range from 1.5 mg/L to 116 mg/L (6-point- calibration), r > 0.99. Plot and equation of the calibration curve is available.

Accuracy

The samples are fortified at 1, 81.6 and 115.1 mg/L

3 recoveries per concentration are determined. Recovery is between 70 and 110 %.

Repeatability

RSD is < 20%

LOQ

1.8 mg/L

Validation is summarized in Table 5.1.2-1

Conclusion

The analytical method is considered acceptable according to SANCO/3030/99 rev. 4 covering the LOQ mentioned.

Table 5.1.2-1: Method Validation for Physical and chemical properties Data

References	Analyte	Detection method	Matrix	LOQ	Fortification level	Mean recovery [%]	RSD [%]	n
KCA 4.1.2/01 Gao (2016)	24-Epibrassinolide	HPLC-UV	0.1% CMC aqueous solution	1.8 mg/L	1.8 mg/L	100	4	3
					81.6 mg/L	102	0.4	3
					115.1 mg/L	98	0.7	3

B.5.1.2.1. *Residue Data*

No studies were submitted/evaluated.

B.5.1.2.2. *Toxicology and Metabolism Data*

Following (non-radiolabelled) analytical methods used in risk assessment studies were evaluated in this chapter.

Reference:	**KCA 5.2/01; ACUTE ORAL TOXICITY STUDY IN RATS WITH 24-EPIBRASSINOLIDE (TGAI)**
Author(s), year:	[redacted] (2017)
Report/Doc. number:	6113 (521-001)
Guideline(s):	-
GLP:	yes

Reference:	**KCA 5.3.2/03; REPEATED DOSE 90 DAYS ORAL TOXICITY STUDY IN RAT WITH 24-EPIBRASSINOLIDE (TGAI)**
Author(s), year:	[redacted] (2017)
Report/Doc. number:	6120 (533-001)
Guideline(s):	-
GLP:	yes

Reference:	**KCA 5.6.2/03; PRENATAL DEVELOPMENTAL TOXICITY STUDY IN WISTAR RATS WITH 24-EPIBRASSINOLIDE (TGAI)**
Author(s), year:	[redacted] (2017)
Report/Doc. number:	6642 (551-001)
Guideline(s):	-
GLP:	yes

All studies above use the same method, but the fortification levels are different. They are assessed together, and the different levels are stated.

Test item

0.1% aqueous Na CMC solution

Analyte

24-Epibrassinolide as derivate (derivatised with phenylboronic acid)

Principle of method

HPLC-UV

Validation

Specificity/Interference

The specificity of the analytical method was verified by checking the retention time of the analyte. Control value is < 30% LOQ

Linearity

A calibration curve is constructed by injecting standard solutions within the range from 200 mg/L to 600 mg/L (5-point- calibration), r > 0.99. Plot and equation of the calibration curve is available.

Accuracy

The samples are fortified at 50 and 100 g/L for KCA 5.2/01

The samples are fortified at 10 and 100 g/L for KCA 5.3.2/03 and KCA 5.6.2/03

5 recoveries per concentration are determined. Recovery is between 70 and 110 %.

Repeatability

RSD is < 20%

LOQ

50 g/L for KCA 5.2/01

10 g/L for for KCA 5.3.2/03 and KCA 5.6.2/03

Validation is summarized in Table 5.1.2-2

Conclusion

The analytical method is considered acceptable covering the LOQ mentioned.

Reference:	**KCA 5.2.3/01; ACUTE INHALATION TOXICITY STUDY IN RATS WITH 24-EPIBRASSINOLIDE (TGAI)**
Author(s), year:	[redacted] (2017)
Report/Doc. number:	6118 (523-001)
Guideline(s):	-
GLP:	yes

Test item

Air sample analysis filters (silica), extracted with acetone

Analyte

24-Epibrassinolide as derivate (derivatised with phenylboronic acid)

Principle of method

HPLC-UV

Validation

Specificity/Interference

The specificity of the analytical method was verified by checking the retention time of the analyte. Control value is < 30% LOQ

Linearity

A calibration curve is constructed by injecting standard solutions within the range from 0.02 mg/L to 25 mg/L (6-point- calibration), r > 0.99. Plot and equation of the calibration curve is available.

Accuracy

The samples are fortified at 0.02 and 0.2 g/L. 5 recoveries per concentration are determined. Recovery is between 70 and 110 %.

Repeatability

RSD is < 20%

LOQ

0.02 g/L

Validation is summarized in Table 5.1.2-2

Conclusion

The analytical method is considered acceptable according to SANCO/3029/99 rev. 4 covering the LOQ mentioned.

Table 5.1.2-2: Method Validation for Toxicology and Metabolism Data

References	Analyte	Detection method	Matrix	LOQ	Fortification level	Mean recovery [%]	RSD [%]	n
KCA 5.2/01 [redacted] (2017)	24-Epibrassinolide	HPLC-UV	0.1% CMC aqueous solution	50 g/L	50 g/L	99	0.4	5
					100 g/L	98	0.03	5
KCA 5.3.2/03 [redacted] (2017) **KCA 5.6.2/03** [redacted] (2017)	24-Epibrassinolide	HPLC-UV	0.1% CMC aqueous solution	10 g/L	10 g/L	99	0.5	5
					100 g/L	99	0.1	5
KCA 5.2.3/01 [redacted] (2017)	24-Epibrassinolide	HPLC-UV	Air sample analysis filters (silica), extracted with acetone	0.02 g/L	0.02 g/L	90	0.7	5
					0.2 g/L	93	0.5	5

B.5.1.2.3. *Environmental Fate And Behaviour*

No studies were submitted/evaluated.

B.5.1.2.4. *Ecotoxicological Data*

Following (non-radiolabelled) analytical methods used in risk assessment studies were evaluated in this chapter.

Reference:	**KCA 8.2.1/01; Acute Fish Toxicity Study in Freshwater Fish (Brachydanio rerio) with 24-Epibrassinolide (TGAI)**
Author(s), year:	[redacted] (2017)
Report/Doc. number:	6122 (821-001)
Guideline(s):	SANCO 3029/99 rev. 4
GLP:	yes

Test item

water

Analyte

24-Epibrassinolide as derivate (derivatised with phenylboronic acid)

Principle of method

HPLC-UV

Validation

Specificity/Interference

The specificity of the analytical method was verified by checking the retention time of the analyte. Control value is < 30% LOQ

Linearity

A calibration curve is constructed by injecting standard solutions within the range from 0.02 mg/L to 25 mg/L (6-point- calibration), r > 0.99. Plot and equation of the calibration curve is available.

Accuracy

The samples are fortified at 0.02 and 0.2 g/L. 5 recoveries per concentration are determined. Recovery is between 70 and 110 %.

Repeatability

RSD is < 20%

LOQ

0.02 g/L

Validation is summarized in Table 5.1.2-3

Conclusion

The analytical method is considered acceptable covering the LOQ mentioned.

Reference:	**KCA 8.2.4.1/01; Acute Toxicity of 24-Epibrassinolide to *Daphnia magna* Under Static Conditions**
Author(s), year:	Matlock, D., Moore, S. (2017)
Report/Doc. number:	115SRUS16C0107 (822-001)
Guideline(s):	SANCO 3029/99 rev. 4
GLP:	yes

Test item

water

Analyte

24-Epibrassinolide as derivate (derivatised with phenylboronic acid)

Principle of method

HPLC-UV

Validation

Specificity/Interference

The specificity of the analytical method was verified by checking the retention time of the analyte. Control value is < 30% LOQ

Linearity

A calibration curve is constructed by injecting standard solutions within the range from 50 µg/L to 400 µg/L (6-point- calibration), r > 0.99. Plot and equation of the calibration curve is available.

Accuracy

The samples are fortified at 0.1, 4 and 5 µg/L. 5 recoveries per concentration are determined. Recovery is between 70 and 110 %.

Repeatability

RSD is < 20%

LOQ

0.1 µg/L

Validation is summarized in Table 5.1.2-3

Conclusion

The analytical method is considered acceptable covering the LOQ mentioned.

Table 5.1.2-3: Method Validation for Ecotoxicological Data

References	Analyte	Detection method	Matrix	LOQ	Fortification level	Mean recovery [%]	RSD [%]	n
KCA 8.2.1/01 Gayathri V. (2017)	24-Epibrassinolide	HPLC-UV	water	0.02 g/L	0.02 g/L	94	3	5
					0.2 g/L	96	1	5
KCA 8.2.4.1/01 Matlock, D., Moore, S. (2017)	24-Epibrassinolide	HPLC-UV	water	0.1 µg/L	0.1 µg/L	95	2	5
					4 µg/L	100	4	5
					5 µg/L	100	3	5

B.5.2. METHODS FOR POST-APPROVAL CONTROL AND MONITORING PURPOSES (CA 4.2)

B.5.2.1. Plant matrices

No residue definition is proposed for 24-Epibrassinolide. Therefore no methods in plant matrices are required.

B.5.2.2. Animal matrices

No residue definition is proposed for 24-Epibrassinolide. Therefore no methods in animal matrices are required.

B.5.2.3. Soil

No residue definition is proposed for 24-Epibrassinolide. Therefore no method in soil is required.

B.5.2.4. Water

No residue definition is proposed for 24-Epibrassinolide. Therefore no method in water is required.

B.5.2.5. Air

No residue definition is proposed for 24-Epibrassinolide. Therefore no method in air is required.

B.5.2.6. Body fluids and tissues

No residue definition is proposed for 24-Epibrassinolide. Therefore no method in body fluids/tissues is required.

B.5.3. REFERENCES 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	Data protection claimed Y/N	Justification if data protection is claimed	Owner	Previous evaluation
KCA 4.1.1/01	Gao, J.	2015	PRELIMINARY ANALYSIS AND ENFORCEMENT ANALYTICAL METHOD OF 24-EPIBRASSINOLIDE TGAI Doc. No.: 172-001 (NC-2015-032) Nutrichem Laboratory Co., Ltd., Beijing, China GLP, unpublished	N	Y	New study necessary for the approval of 24-Epibrassinolide	Suntton GmbH	N