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ISOFLUCYPRAM

Volume 3 – B.5 (PPP) – Isoflucypram EC 50

Rapporteur Member State : United Kingdom
Co-Rapporteur Member State : France

Version History

When	What
March 2019	Initial DAR

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B.5. METHODS OF ANALYSIS

B.5.1. METHODS USED FOR THE GENERATION OF PRE-AUTHORISATION DATA

B.5.1.1. Analysis of the plant protection product

B.5.1.1.1. *Method for the determination of the active substance in the plant protection product.*

Report:	KCP 5.1.1/01; Michel, A.; 2017; M-507982-02-1
Title:	Determination of isoflucypram in formulations - Assay - HPLC, external standard
Report No.:	AM027115MF2
Document No.:	M-507982-02-1
Guideline(s):	REGULATION (EC) No 1107/2009, Commission Regulation 545/2011, 5.1, US EPA OCSPP 830.1800
Guideline deviation(s):	not specified
GLP/GEP:	**no**

Report:	KCP 5.1.1/02; Michel, A; Garcia Sanchez, M. T.; 2017; M-597272-01-1
Title:	Validation of Analytical Method AM027115MF2 - Determination of isoflucypram in the formulation isoflucypram EC 50 (50 g/L) - Final Report -
Report No.:	VB1-AM027115MF2
Document No.:	M-597272-01-1
Guideline(s):	SANCO/3030/99 rev. 4, Commission Regulation (EU) 284/2013 in accordance with Regulation (EC) No 1107/2009, US EPA OCSPP Test Guideline No. 830.1800
Guideline deviation(s):	Not specified
GLP/GEP:	**yes**

Method AM027115MF1 was used to determine the content of Isoflucypram in the plant protection product Isoflucypram EC 50 (50 g/L) during the storage stability studies described in Volume 3CP, Section B.2.7. The applicant has confirmed that methods AM027115MF1 and AM027115MF2 are different versions of the same method and that the later version was created to 'correct typing errors' relating to the chromatographic conditions. They have confirmed that 'all testing (including accelerated and cold storage stability) have been performed according to the validated parameters in AM027115MF2'.

Full details of the method AM027115MF2 and its validation are presented below and in Table B.5.1.1-1.

Principle of the method
Samples of test item are dissolved in acetonitrile with the aid of ultrasonication then diluted with water to give a final solvent composition of approximately 1 :1 acetonitrile/water. Any turbidity was removed by centrifugation before the samples were analysed by reverse phase HPLC-UV (220 nm) using a Waters Symmetry C18, 100mm x 4.6mm x 3.5µm analytical column at 40°C and gradient elution with acetonitrile and 0.01 M aqueous phosphoric acid mobile phases. Quantification was against external standards.

Specificity:
Specificity was demonstrated by retention time match with a reference standard and the absence of interfering peaks in the chromatogram of a blank formulation sample.

Linearity:
Linearity was demonstrated over two ranges by the analysis of two sets of six/seven standards of increasing concentration. The ranges of standard concentrations used were equivalent to 2.6 - 26 g/L (n=7) and 26 – 78 g/L (n=6) in the formulation. The higher range of concentrations adequately encompasses the concentration of isoflucypram in the formulation (50 g/L). The correlation coefficients were >0.99.

Accuracy:
Six recovery samples of increasing concentrations were prepared by spiking blank formulation with Isoflucypram standard and analysing them by the method described. The spike concentrations were in the range 26 – 78 g/L (isoflucypram in formulation), which is 50 - 150% of the nominal concentration of 50 g/L. Recovery values were calculated as a percentage of measured concentration relative to fortified concentration. Acceptable mean recovery levels are within the range 97 to 103 %.

Precision
Six replicates samples were prepared using the method described above and the %RSD was calculated. Acceptable precision (repeatability) at the nominal concentration of 50 g/L (~5 %w/w) is 2.1%.

Assessment
The method is acceptably validated in accordance with SANCO/3030/99 rev.4 and is suitable for the determination of isoflucypram in Isoflucypram EC 50 (50 g/L)

Table B.5.1.1-1 Summary of validation data for analytical method AM027115MF2 for the determination of BCS-CN88460 in Isoflucypram EC 50 (50 g/L).

Analyte	Recovery fortification level (g/L)	% Recovery	Repeatability % RSD (n)	Linearity	Specificity
BCS-CN88460	25 29 40 50 62 75	99.6 99.6 99.5 99.4 99.9 99.3	0.22 @ ~50 g/L (n=6) Modified Horwitz = 2.1 @ 5 %w/w	0.012 to 0.37 mg/ml (26 to 78 g/L) y = 0.7621x - 0.2906 r = 0.99990 0.2 to 0.12 mg/ml (2.6 to 26 g/L) y = 0.7591x - 0.1247 r = 0.99998	Retention time match to reference standard. No significant interfering peaks observed in the blank formulation.

Nominal content in the formulation is 50 g/L.

B.5.1.1.2. *Methods for the determination of relevant impurities identified in the technical material or which may be formed during manufacture of the plant protection product or from degradation of the plant protection product during storage*

Report:	KCP 5.1.1/03; Schulz, F.; 2018; M-615231-01-1
Title:	Determination of impurity BCS-CN45153 in formulations containing isoflucypram - Assay - HPLC-MS/MS, external standard
Report No.:	AM031618MF1
Document No.:	M-615231-01-1
Guideline(s):	Commission Regulation (EU) 284/2013 in accordance with Regulation (EC) No 1107/2009 (10/2009), US EPA OCSPP Test Guideline No. 830.1800 (08/1996)
Guideline deviation(s):	Not specified
GLP/GEP:	**no**

Report:	KCP 5.1.1/04; Schulz, F.; Garcia Sanchez, M. T.; 2018; M-617266-01-1
Title:	Validation of Analytical Method AM031618MF1 - Determination of BCS-CN45153 in the formulation isoflucypram EC 50 (50 g/L) - Final Report -
Report No.:	VB1-AM031618MF1
Document No.:	M-617266-01-1
Guideline(s):	SANCO/3030/99 rev. 4 (07/2000), Commission Regulation (EU) 284/2013 (03/2013) in accordance with Regulation (EC) No 1107/2009 (10/2009), US EPA OCSPP Test Guideline No. 830.1800 (08/1996)
Guideline deviation(s):	Not specified
GLP/GEP:	**yes**

The content of BCS-CN45153 in the plant protection product Isoflucypram EC 50 (50 g/L) is determined using analytical method AM031618MF1. Full details of the method and its validation are presented below and in Table B.5.1.1-2

Principle of the method
Samples of test item are dissolved in solvent mixture (0.1 mL formic acid in 1L acetonitrile/water 80 :20 v/v) with the aid of ultrasonication then made up to a fixed volume with more of the solvent mixture. Any turbidity is removed by centrifugation, then an aliquot of the clear extract is diluted with further solvent mixture before analysis by reverse phase HPLC-MS-MS using a Zorbax SB RP18, 75mm x 2.1mm x 3.5µm analytical column at 40ºC and gradient elution with water (+ 0.01% formic acid) and acetonitrile (+0.01% formic acid) mobile phases. Two ion transitions were monitored; m/z 365.8→105.1 for quantification and 365.8→133.1 for confirmation. Quantification was against external standards.

Specificity:
Specificity was demonstrated by retention time match with a reference standard and the absence of interfering peaks in the chromatogram of a blank formulation sample.

Linearity:
Linearity was demonstrated by the analysis of seven standards of increasing concentration. The standard concentrations used ranged from 5 to 120% of maximum permitted content of BCS-CN45153 in the formulation of 0.05 g/L (based on a maximum specified value of 1 g/kg in the technical material and a formulation concentration of 50 g/L). The correlation coefficient was >0.99.

Accuracy:
Blank formulation was fortified with reference standards at each of two levels, 0.05 and 0.005 g/L, which approximately equate to the maximum permitted content of BCS-CN45153 in the formulation and also 10 times less than the maximum. Six replicates were analysed at each level. Recovery values were calculated as a percentage of measured concentration relative to fortified concentration. Acceptable mean recovery levels are within the range 75 to 125 %.

Precision:

Six replicates samples were prepared using the method described above and the %RSD was calculated.
Confirmation:
Overlayed chromatograms obtained for the quantification and confirmation transistions were submitted for both reference standard and sample solutions, demonstrating that the confirmatory transition is suitable for qualitative confirmation of identity.

Assessment:
The method is acceptably validated in accordance with SANCO/3030/99 rev.4 and is suitable for the determination of BCS-CN45153 in Isoflucypram EC 50 (50 g/L) at an LOQ of 0.005 g/L, which is approximately ten-fold lower than the maximum permitted content of BCS-CN45153 in the formulation of 0.05 g/L (based on a maximum specified value of 1 g/kg in the technical material and a formulation concentration of 50 g/L).

Table B.5.1.1-1 Summary of validation data for analytical method AM031618MF1 for the determination of BCS-CN45153 in Isoflucypram EC 50 (50 g/L).

Analyte	Recovery fortification level (g/L)	% Recovery range (mean)	Repeatability % RSD (n)	Linearity	Specificity
BCS-CN45153	0.00505	89 – 99 (92) %RSD = 4.4 (n=6) Modified Horwitz = 8.4 @ 0.0005 %w/w	3.5 @ ~0.04 g/L (n=6) Modified Horwitz = 6.2 @ 0.004 %w/w	0.00053 to 0.012 µg/ml (0.0026 to 0.06 g/L) y = 195144817x + 12705 r = 0.99983	Retention time match to reference standard. No significant interfering peaks observed in the blank formulation.
	0.0528	89 – 108 (95) %RSD = 7.7 (n=6) Modified Horwitz = 5.9 @ 0.005 %w/w			

Maximum content of BCS-CN45153 in the formulation is 0.05 g/L.

B.5.1.1.3. *Methods for the determination of relevant co-formulants or components of co-formulants, where required by the national competent authorities*

With respect to toxicological, eco-toxicological or environmental aspects the product does not contain any relevant formulants. Therefore, a special analytical method and validation is not needed.

B.5.1.2. Methods for the determination of residues

B.5.1.2.1. *Methods in soil, water, sediment, air and any additional matrices used in support of environmental fate studies*

Refer to Volume 3CA, Section B.5.1.2.1.

B.5.1.2.2. *Methods in soil, water and any additional matrices in support of efficacy studies*

Refer to Volume 3CA, Section B.5.1.2.2.

B.5.1.2.3. *Methods in feed, body fluids and tissues, air and any additional matrices used in support of toxicological studies*

Refer to Volume 3CA, Section B.5.1.2.3.

B.5.1.2.4. *Methods in body fluids, air, and any additional matrices used in support of operator, worker, resident and bystander exposure studies*

Refer to Volume 3CA, Section B.5.1.2.4.

B.5.1.2.5. *Methods in or on plants, plant products, processed food commodities, food of plant and animal origin, feed and any additional matrices used in support of residues studies*

Refer to Volume 3CA, Section B.5.1.2.5.

B.5.1.2.6. *Methods in soil, water, sediment, feed and any additional matrices used in support of ecotoxicology studies*

Refer to Volume 3CA, Section B.5.1.2.6.

B.5.1.2.7. *Methods in water, buffer solutions, organic solvents and any additional matrices resulting from the physical and chemical properties tests*

Refer to Volume 3CA, Section B.5.1.2.7.

B.5.2. METHODS FOR POST-APPROVAL CONTROL AND MONITORING PURPOSES

B.5.2.1. Analysis of the plant protection product

Refer to section B.5.1.1 above.

B.5.2.2. Methods for the determination of residues in/on food and feed of plant origin

Refer to Volume 3CA, Sections B.5.2.1

B.5.2.3. Methods for the determination of residues in/on food and feed of animal origin

Refer to Volume 3CA, Sections B.5.2.2

B.5.2.4. Methods for the determination of residues in soil

Refer to Volume 3CA, Section B.5.2.3

B.5.2.5. Methods for the determination of residues in water

Refer to Volume 3CA, Section B.5.2.4

B.5.2.6. Methods for the determination of residues in air

Refer to Volume 3CA, Section B.5.2.5

B.5.2.7. Methods for the determination of residues in body fluids and tissues

Refer to Volume 3CA, Section B.5.2.6

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
KCP 5.1.1 / 01	Michel, A.	2017	Determination of isoflucypram in formulations - Assay - HPLC, external standard Bayer AG, Crop Science Division, Monheim, Germany Bayer Report No.: AM027115MF2 Edition Number: M-507982-02-1 Date: 2015-01-19 **... amended: 2017-05-02** GLP/GEP: No, unpublished	No	No		Bayer	N/A
KCP 5.1.1 / 02	Michel, A; Garcia Sanchez, M. T.	2017	Validation of Analytical Method AM027115MF2 - Determination of isoflucypram in the formulation isoflucypram EC 50 (50 g/L) - Final Report - Bayer AG, Crop Science Division, Monheim, Germany Bayer Report No.: VB1-AM027115MF2 Edition Number: M-597272-01-1 Date: 2017-07-27 GLP/GEP: Yes, unpublished	No	Yes	New data for a new active substance	Bayer	N/A
KCP 5.1.1 / 03	Schulz, F.	2018	Determination of impurity BCS-CN45153 in formulations containing isoflucypram - Assay - HPLC-MS/MS, external standard Bayer AG, Crop Science Division, Monheim, Germany Bayer Report No.: AM031618MF1 Edition Number: M-615231-01-1 Date: 2018-02-21 GLP/GEP: No, unpublished	No	No		Bayer	N/A

KCP 5.1.1 / 04	Schulz, F.; Garcia Sanchez, M. T.	2018	Validation of Analytical Method AM031618MF1 - Determination of BCS-CN45153 in the formulation isoflucypram EC 50 (50 g/L) - Final Report - Bayer AG, Crop Science Division, Monheim, Germany Bayer Report No.: VB1-AM031618MF1 Edition Number: M-617266-01-1 Date: 2018-03-12 GLP/GEP: Yes, unpublished	No	Yes	New data for a new active substance	Bayer	N/A